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MOOD DISORDER GENE

The invention is concerned with the determination
 of genetic factors associated with psychiatric health
 with particular reference to a human gene or genes
 which contributes to or is responsible for the
 manifestation of a mood disorder or a related disorder
 in affected individuals. In particular, although not
 exclusively, the invention provides a method of
 identifying and characterising such a gene or genes
 from human chromosome 18, as well as genes so
 identified and their expression products. The
 invention is also concerned with methods of
 determining the genetic susceptibility of an
 individual to a mood disorder or related disorder. By
 mood disorders or related disorders is meant the
 following disorders as defined in the Diagnostic and
 Statistical Manual of Mental Disorders, version 4
 (DSM-IV) taxonomy (DSM-IV codes in parenthesis):- mood
 disorders (296.XX, 300.4, 311, 301.13, 295.70),
 schizophrenia and related disorders (295.XX,
 297.1, 298.8, 297.3, 298.9), anxiety disorders (300.XX,
 309.81, 308.3), adjustment disorders (309.XX) and
 personality disorders (codes 301.XX).

The methods of the invention are particularly
 exemplified in relation to genetic factors associated
 with a family of mood disorders known as Bipolar (BP)
 spectrum disorders.

Bipolar disorder (BP) is a severe psychiatric
 condition that is characterized by disturbances in
 mood, ranging from an extreme state of elation (mania)
 to a severe state of dysphoria (depression). Two types
 of bipolar illness have been described: type I BP
 illness (BPI) is characterized by major depressive
 episodes alternated with phases of mania, and type II

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BP illness (BPII), characterized by major depressive episodes alternating with phases of hypomania. Relatives of BP probands have an increased risk for BP, unipolar disorder (patients only experiencing depressive episodes; UP), cyclothymia (minor depression and hypomania episodes; CY) as well as for schizoaffective disorders of the manic (SAM) and depressive (SAD) type. Based on these observations BP, CY, UP and SA are classified as BP spectrum disorders.

The involvement of genetic factors in the etiology of BP spectrum disorders was suggested by family, twin and adoption studies (Tsuang and Faraone (1990), The Genetics of Mood Disorders, Baltimore, The John Hopkins University Press). However, the exact pattern of transmission is unknown. In some studies, complex segregation analysis supports the existence of a single major locus for BP (Spence et al. (1995), Am J. Med. Genet (Neuropsych. Genet.) 60 pp 370-376). Other researchers propose a liability-threshold-model, in which the liability to develop the disorder results from the additive combination of multiple genetic and environmental effects (McGuffin et al. (1994), Affective Disorders; Seminars in Psychiatric Genetics Gaskell, London pp 110-127).

Due to the complex mode of inheritance, parametric and nonparametric linkage strategies are applied in families in which BP disorder appears to be transmitted in a Mendelian fashion. Early linkage findings on chromosomes 11p15 (Egeland et al. (1987), Nature 325 pp 783-787) and Xq27-q28 (Mendlewicz et al. (1987) The Lancet 1 pp 1230 -1232; Baron et al. (1987) Nature 326 pp 289-292) have been controversial and could initially not be replicated (Kelsoe et al. (1989) Nature 242 pp 238-243; Baron et al. (1993) Nature Genet 3 pp 49-55). With the development of a

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human genetic map saturated with highly polymorphic markers and the continuous development of data analysis techniques, numerous new linkage searches were started. In several studies, evidence or
5 suggestive evidence for linkage to particular regions on chromosomes 4, 12, 18, 21 and X was found (Blackwood et al. (1996) Nature Genetics 12 pp 427-430, Craddock et al. (1994) Brit J. Psychiatry 164 pp 355-358, Berrettini et al. (1994), Proc Natl Acad Sci
10 USA 91 pp 5918-5921, Straub et al. (1994) Nature Genetics 8 pp 291-296 and Pekkarinen et al. (1995) Genome Research 5 pp 105-115). In order to test the validity of the reported linkage results, these findings have to be replicated in other, independent
15 studies.

Recently, linkage of bipolar disorder to the pericentromeric region on chromosome 18 was reported (Berrettini et al. 1994). Also a ring chromosome 18 with break-points and deleted regions at 18pter-p11
20 and 18q23-qter was reported in three unrelated patients with BP illness or related syndromes (Craddock et al. 1994). The chromosome 18p linkage was replicated by Stine et al. (1995) Am J Hum Genet
25 57 pp 1384-1394, who also reported suggestive evidence for a locus on 18q21.2-q21.32 in the same study. Interestingly, Stine et al. observed a parent-of-origin effect: the evidence of linkage was the strongest in the paternal pedigrees, in which the proband's father or one of the proband's father's sibs
30 is affected.

In an independent replication study, the present inventors tested linkage with chromosome 18 markers in
10 Belgian families with a bipolar proband. To localize causative genes the linkage analysis or
35 likelihood method was used in these families. This

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method studies within a family the segregation of a defined disease phenotype with that of polymorphic genetic markers distributed in the human genome. The likelihood ratio of observing cosegregation of the disease and a genetic marker under linkage versus no linkage is calculated and the log of this ratio or the log of the odds is the LOD score statistic z . A LOD score of 3 (or likelihood ratio of 1000 or greater) is taken as significant statistical evidence for linkage. In the inventors' study no evidence for linkage to the pericentromeric regions was found, but in one of the families, MAD31, a Belgian family of a BPII proband, suggestive linkage was found with markers located at 18q21.33-q23 (De bruyn et al. (1996) Biol Psychiatry 39 pp 679-688). Multipoint linkage analysis gave the highest LOD score in the interval between STR (Short Tandem Repeats) polymorphisms D18S51 and D18S61, with a maximum multipoint LOD score of +1.34. Simulation studies indicated that this LOD score is within the range of what can be expected for a linked marker given the information available in the family. Likewise, an affected sib-pair analysis also rejected the null-hypothesis of nonlinkage for several of the markers tested. Two other groups also found evidence for linkage of bipolar disorder to 18q (Freimer et al. (1996) Nature Genetics 12 pp 436-441, Coon et al. (1996) Biol Psychiatry 39 pp 689 to 696). Although the candidate regions in the different studies do not entirely overlap, they all suggest the presence of a susceptibility locus at 18q21-q23.

The inventors have now carried out further investigations into the 18q chromosomal region in family MAD31. By analysis of cosegregation of bipolar disease in MAD31 with twelve STR polymorphic markers previously located between the aforementioned markers

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D18S51 and D18S61 and subsequent LOD score analysis as described above, the inventors have further refined the candidate region of chromosome 18 in which a gene associated with mood disorders such as bipolar spectrum disorders may be located and have constructed a physical map. The region in question may thus be used to locate, isolate and sequence a gene or genes which influences psychiatric health and mood.

The inventors have also constructed a YAC (yeast artificial chromosome) contig map of the candidate region to determine the relative order of the twelve STR markers mapped by the cosegregational analysis and they have identified seven clones from the YAC library incorporating the candidate region.

A number of procedures can be applied to the identified YAC clones and, where applicable, to the DNA of an individual afflicted with a mood disorder as defined herein, in the process of identifying and characterising the relevant gene or genes. For example, the inventors have used YAC clones spanning the region of interest in chromosome 18 to identify by CAG or CTG fragmentation novel genes that are allegedly involved in the manifestation of mood disorders or related disorders.

Other procedures can also be applied to the said YAC clones to identify candidate genes as discussed below.

Once candidate genes have been identified it is possible to assess the susceptibility of an individual to a mood disorder or related disorder by detecting the presence of a polymorphism associated with a mood disorder or related disorder in such genes.

Accordingly, in a first aspect the present invention comprises the use of an 8.9 cM region of

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human chromosome 18q disposed between polymorphic markers D18S68 and D18S979 or a fragment thereof for identifying at least one human gene, including mutated and polymorphic variants thereof, which is associated with mood disorders or related disorders as defined above. As will be described below, the present inventors have identified this candidate region of chromosome 18q for such a gene, by analysis of cosegregation of bipolar disease in family MAD31 with 12 STR polymorphic markers previously located between D18S51 and D18S61 and subsequent LOD score analysis.

In a second aspect the invention comprises the use of a YAC clone comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61 for identifying at least one human gene, including mutated or polymorphic variants thereof, which is associated with mood disorders or related disorders as defined above. D18S60 is close to D18S51 so the particular YAC clones for use are those which have an artificial chromosome spanning the candidate region of human chromosome 18q between polymorphic markers D18S51 and D18S61 as identified by the present inventors in their earlier paper (De bruyn et al. (1996)).

Particular YACs covering the candidate region which may be used in accordance with the present invention are 961.h.9, 942.c.3, 766.f.12, 731.c.7, 907.e.1, 752-g-8 and 717.d.3, preferred ones being 961.h.9, 766.f.12 and 907.e.1 since these have the minimum tiling path across the candidate region. Suitable YAC clones for use are those having an artificial chromosome spanning the refined candidate region between D18S68 and D18S979.

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There are a number of methods which can be applied to the candidate regions of chromosome 18q as defined above, whether or not present in a YAC, to identify a candidate gene or genes associated with mood disorders or related disorders. For example, it has previously been demonstrated that an apparent association exists between the presence of trinucleotide repeat expansions (TRE) in the human genome and the phenomenon of anticipation of mood disorders (Lindblad et al. (1995), Neurobiology of Disease 2: 55-62 and O'Donovan et al. (1995), Nature Genetics 10: 380-381).

Accordingly, in a third aspect the present invention comprises a method of identifying at least one human gene, including mutated and polymorphic variants thereof, which is associated with a mood disorder or related disorder as defined herein which comprises detecting nucleotide triplet repeats in the region of human chromosome 18q disposed between polymorphic markers D18S68 and D18S979.

An alternative method of identifying said gene or genes comprises fragmenting a YAC clone comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61, for example one or more of the seven aforementioned YAC clones, and detecting any nucleotide triplet repeats in said fragments. Nucleic acid probes comprising at least 5 and preferably at least 10 CTG and/or CAG triplet repeats are a suitable means of detection when appropriately labelled. Trinucleotide repeats may also be determined using the known RED (repeat expansion detection) system (Shalling et al. (1993), Nature Genetics 4 pp 135-139).

In a fourth embodiment the invention comprises a

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method of identifying at least one gene, including mutated and polymorphic variants thereof, which is associated with a mood disorder or related disorder and which is present in a YAC clone spanning the region of human chromosome 18q between polymorphic markers D18S60 and D18S61, the method comprising the step of detecting the expression product of a gene incorporating nucleotide triplet repeats by use of an antibody capable of recognising a protein with an amino acid sequence comprising a string of at least 8, but preferably at least 12, continuous glutamine residues. Such a method may be implemented by subcloning YAC DNA, for example from the seven aforementioned YAC clones, into a human DNA expression library. A preferred means of detecting the relevant expression product is by use of a monoclonal antibody, in particular mAB 1C2, the preparation and properties of which are described in International Patent Application Publication No WO 97/17445.

As will be described in detail below, in order to identify candidate genes containing triplet repeats, the inventors have carried out direct CAG or CTG fragmentation of YACs 961.h.9, 766.f.12 and 907.e.1, comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61, and have identified a number of sequences containing CAG or CTG repeats, whose abnormal expansion may be involved in genetic susceptibility to a mood disorder or related disorder.

Accordingly, in a fifth aspect, the invention provides a nucleic acid comprising the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a.

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In a further aspect, the invention provides a protein comprising an amino acid sequence encoded by the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a.

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In yet a further aspect the invention provides a mutated nucleic acid comprising a sequence of nucleotides which differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a only in the extent of trinucleotide repeats.

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Also provided by the invention is a mutated protein comprising an amino acid sequence encoded by a sequence of nucleotides which differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a only in the extent of trinucleotide repeats.

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It is to be understood that the invention also contemplates nucleotide sequences having at least 75% and preferably at least 80% homology with any of the sequences described above and having functional identity with any of said sequences. The homology is calculated as described by Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402, Karlin et al. (1990) Proc Natl Acad Sci USA 87: 2264-68 and Karlin et al. (1993) Proc Natl Acad Sci USA 90: 5873-5877. Also contemplated are amino acid sequences which differ from the above described sequences only in conservative amino acid changes. Suitable changes are well known to those skilled in the art.

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Knowledge of the sequences described above can be used to design assays to determine the genetic susceptibility of an individual to a mood disorder or

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related disorder.

Accordingly, in a further aspect the invention provides a method for determining the susceptibility of an individual to a mood disorder or related
5 disorder which comprises the steps of:

a) obtaining a DNA sample from said individual;

10 b) providing primers suitable for the amplification of a nucleotide sequence comprised in the sequence shown in any one of Figures 15a, 16a, 17a or 18a said primers flanking the trinucleotide repeats comprised in said sequence;

15 c) applying said primers to the said DNA sample and carrying out an amplification reaction;

20 d) carrying out the same amplification reaction on a DNA sample from a control individual; and

e) comparing the results of the amplification reaction for the said individual and for
25 the said control individual;

30 wherein the presence of an amplified fragment from said individual which is bigger in size from that of said control individual is an indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.

By control individual is meant an individual who is not affected by a mood disorder or related disorder and does not have a family history of mood disorders
35 or related disorders.

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Preferable primers to use in this method are those shown in Figure 15b, 16b, 17b or 18b but other suitable primers may be utilised.

5 In a further aspect the invention provides a method of determining the susceptibility of an individual to a mood disorder or related disorder which method comprises the steps of :

10 a) obtaining a protein sample from said individual; and

 b) detecting the presence of a protein comprising an amino acid sequence encoded by a
15 sequence of nucleotides which differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a only in the extent of trinucleotide repeats

20 wherein the presence of said protein is an indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.

 Preferably, the foresaid protein is detected by
 utilising an antibody that is capable of recognising a
25 string of at least 8 continuous glutamines as, for example, the mAB 1C2 antibody.

 The nucleic acids molecules according to the invention may be advantageously included in an
30 expression vector, which may be introduced into a host cell of prokaryotic or eukaryotic origin. Suitable expression vectors include plasmids, which may be used to express foreign DNA in bacterial or eukaryotic host cells, viral vectors, yeast artificial chromosomes or
35 mammalian artificial chromosomes. The vector may be

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transfected or transformed into host cells using suitable methods known in the art such as, for example, electroporation, microinjection, infection, lipoinfection and direct uptake. Such methods are described in more detail, for example, by Sambrook et al., "Molecular Cloning: A Laboratory Manual", 2nd ed. (1989) and by Ausbel et al. "Current Protocols in Molecular Biology", (1994).

Also provided by the invention is a host cell, tissue or organism comprising the expression vector according to the invention. The invention further provides a transgenic host cell, tissue or organism comprising a transgene capable of encoding the proteins of the invention, which may comprise a genomic DNA or a cDNA. The transgene may be present in the transgenic host cell, tissue or organism either stably integrated into the genome or in an extra chromosomal state.

A nucleic acid molecule comprising a nucleotide sequence shown in any one of Figures 15a, 16a, 17a or 18a as well as the protein encoded by it may be therapeutically used in the treatment of mood disorders or related disorders in patients which present a trinucleotide repeat expansion (TRE) in at least one of the foresaid sequences.

Accordingly, in another of its aspects the invention provides the above described nucleic acid molecules and proteins for use as medicaments for the treatment of individuals with a mood disorder or related disorder. Preferably, the nucleic acid or the protein is present in an appropriate carrier or delivery vehicle. As an example, the nucleic acid inserted into a vector, for example a plasmid or a

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viral vector, may be transfected into a mammalian cell such as a somatic cell or a mammalian germ line cell, as described above. The cell to be transfected can be present in a biological sample obtained from the patient, for example blood or bone marrow, or can be obtained from cell culture. After transfection the sample may be returned or readministered to a patient according to methods known to those practised in the art, for example, methods as described in Kasid et al., Proc. Natl. Acad. Sci. USA (1990) 87:473; Rosenberg et al. (1990) New Eng. J. Med. 323: 570 ; Williams et al. (1994) Nature 310: 476; Dick et al. (1985) Cell 42:71; Keller et al. (1985) Nature 318: 149 and Anderson et al. (1994) US Patent N. 5,399,346.

15 There are a number of viral vectors known to
those skilled in the art which can be used to
introduce the nucleic acid into mammalian cells, for
example retroviruses, parvoviruses, coronaviruses,
negative strand RNA viruses such as picornaviruses or
20 alphaviruses and double stranded DNA viruses including
adenoviruses, herpesviruses such as Herpes Simplex
virus types 1 and 2, Epstein-Barr virus or
cytomegalovirus and poxviruses such as vaccinia
fowlpox or canarypox. Other viruses include, for
25 example, Norwalk viruses, togaviruses, flaviviruses,
reoviruses, papovaviruses, hepadnaviruses and
hepatitis viruses.

A preferred method to introduce nucleic acid that encodes the desired protein into cells is through the use of engineered viral vectors. These vectors provide a means to introduce nucleic acids into cycling and quiescent cells and have been modified to reduce cytotoxicity and to improve genetic stability. The preparation and use of engineered Herpes simplex virus type 1 (D.M. Krisky, et al. (1997) Gene Therapy

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4(10): 1120-1125), adenoviral (A. Amalfitano, et al. (1998) Journal of Virology 72(2):926-933), attenuated lentiviral (R. Zufferey, et al., Nature Biotechnology (1997) 15(9):871-875) and
5 adenoviral/retroviral chimeric (M. Feng, et al, Nature Biotechnology (1997) 15(9):866-870) vectors are known to the skilled artisan.

The protein may be administered using methods known in the art. For example, the mode of
10 administration is preferably at the location of the target cells. The administration can be by injection. Other modes of administration (parenteral, mucosal, systemic, implant, intraperitoneal, etc.) are generally known in the art. The agents can,
15 preferably, be administered in a pharmaceutically acceptable carrier, such as saline, sterile water, Ringer's solution and isotonic sodium chloride solution.

20 In yet another of its aspects the invention provides assay methods for identifying compounds that are able to enhance or inhibit the expression of the proteins of the invention. These assays can be conducted, for example, by transfecting a nucleic acid
25 of the invention into host cells and then comparing the levels of mRNA transcript or the levels of protein expressed from said nucleic acids in the presence or absence of the compound. Different methods, well known to those skilled in the
30 art can be employed in order to measure transcription or expression levels. Alternatively, it is possible to identify compounds that modulate transcription by using a reporter gene assay of the type well known in the art. In such an
35 assay a reporter plasmid is constructed in which the

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promoter of a gene, whose levels of transcription are to be monitored, is positioned upstream of a gene capable of expressing a reporter molecule. The reporter molecule is a molecule whose level of expression can be easily detected and may be either the transcript of the reporter gene or a protein with characteristics that allow it to be detected. For example, the molecule may be a fluorescent protein such as green fluorescent protein (GFP).

Compound assays may be conducted by introducing the reporter plasmid described above into an appropriate host cell and then measuring the amount of reporter molecule expressed in the presence or absence of the compound to be tested.

The invention also relates to compounds identified by the above mentioned methods.

Further embodiments of the present invention relate to methods of identifying the relevant gene or genes which involve the sub-cloning of YAC DNA as defined above into vectors such as BAC (bacterial artificial chromosome) or PAC (P1 or phage artificial chromosome) or cosmid vectors such as exon-trap cosmid vectors. The starting point for such methods is the construction of a contig map of the region of human chromosome 18q between polymorphic markers D18S60 and D18S61. To this end the present inventors have sequenced the end regions of the fragment of human DNA in each of the seven aforementioned YAC clones and these sequences are disclosed herein. Following subcloning of YAC DNA into other vectors as described above, probes comprising these end sequences or portions thereof, in particular those sequences shown in Figures 1 to 11 herein, together with any known

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sequenced tagged site (STS) in this region, as described in the YAC clone contig shown herein, as can be used to detect overlaps between said subclones and a contig map can be constructed. Also the known sequences in the current YAC contig can be used for the generation of contig map subclones.

One route by which a gene or genes which is associated with a mood disorder or associated disorder can be identified is by use of the known technique of exon trapping.

This is an artificial RNA splicing assay, most often making use in current protocols of a specialized exon-trap cosmid vector. The vector contains an artificial minigene consisting of a segment of the SV40 genome containing an origin of replication and a powerful promoter sequence, two splicing-competent exons separated by an intron which contains a multiple cloning site and an SV40 polyadenylation site.

The YAC DNA is subcloned in the exon-trap vector and the recombinant DNA is transfected into a strain of mammalian cells. Transcription from the SV40 promoter results in an RNA transcript which normally splices to include the two exons of the minigene. If the cloned DNA itself contains a functional exon, it can be spliced to the exons present in the vector's minigene. Using reverse transcriptase a cDNA copy can be made and using specific PCR primers, splicing events involving exons of the insert DNA can be identified. Such a procedure can identify coding regions in the YAC DNA which can be compared to the equivalent regions of DNA from a person afflicted with a mood disorder or related disorder to identify the relevant gene.

Accordingly, in a further aspect the invention

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comprises a method of identifying at least one human gene, including mutated variants and polymorphisms thereof, which is associated with a mood disorder or related disorder which comprises the steps of:

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(a) transfecting mammalian cells with exon trap cosmid vectors prepared and mapped as described above;

10 (b) culturing said mammalian cells in an appropriate medium;

(c) isolating RNA transcripts expressed from the SV40 promoter;

15 (d) preparing cDNA from said RNA transcripts;

(e) identifying splicing events involving exons of the DNA subcloned into said exon trap cosmid vectors to elucidate positions of coding regions in
20 said subcloned DNA;

(f) detecting differences between said coding regions and equivalent regions in the DNA of an individual afflicted with said mood disorder or
25 related disorder; and

(g) identifying said gene or mutated or polymorphic variant thereof which is associated with said mood disorder or related disorders.

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As an alternative to exon trapping the YAC DNA may be subcloned into BAC, PAC, cosmid or other vectors and a contig map constructed as described above. There are a variety of known methods available
35 by which the position of relevant genes on the

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subcloned DNA can be established as follows:

5 (a) cDNA selection or capture (also called direct
selection and cDNA selection): this method involves
the forming of genomic DNA/cDNA heteroduplexes by
hybridizing a cloned DNA (e.g. an insert of a YAC
DNA), to a complex mixture of cDNAs, such as the
inserts of all cDNA clones from a specific (e.g.
brain) cDNA library. Related sequences will hybridize
10 and can be enriched in subsequent steps using biotin-
streptavidine capturing and PCR (or related
techniques);

15 (b) hybridization to mRNA/cDNA: a genomic clone
(e.g. the insert of a specific cosmid) can be
hybridized to a Northern blot of mRNA from a panel of
culture cell lines or against appropriate (e.g. brain)
cDNA libraries. A positive signal can indicate the
presence of a gene within the cloned fragment;

20 (c) CpG island identification: CpG or HTF islands
are short (about 1 kb) hypomethylated GC-rich (> 60%)
sequences which are often found at the 5' ends of
genes. CpG islands often have restriction sites for
25 several rare-cutter restriction enzymes. Clustering
of rare-cutter restriction sites is indicative of a
CpG island and therefore of a possible gene. CpG
islands can be detected by hybridization of a DNA
clone to Southern blots of genomic DNA digested with
30 rare-cutting enzymes, or by island-rescue PCR
(isolation of CpG islands from YACs by amplifying
sequences between islands and neighbouring Alu-
repeats);

35 (d) zoo-blotting: hybridizing a DNA clone (e.g.

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the insert of a specific cosmid) at reduced stringency against a Southern blot of genomic DNA samples from a variety of animal species. Detection of hybridization signals can suggest conserved sequences, indicating a possible gene.

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Accordingly, in a further aspect the invention comprises a method of identifying at least one human gene including mutated and polymorphic variants thereof which is associated with a mood disorder or related disorder which comprises the steps of:

10

(a) subcloning the YAC DNA as described above into a cosmid, BAC, PAC or other vector;

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(b) using the nucleotide sequences shown in any one of Figures 1 to 11 or any other sequenced tagged site (STS) in this region as in the YAC clone contig described herein, or part thereof consisting of not less than 14 contiguous bases or the complement thereof, to detect overlaps amongst the subclones and construct a map thereof;

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(c) identifying the position of genes within the subcloned DNA by one or more of CpG island identification, zoo-blotting, hybridization of the subcloned DNA to a cDNA library or a Northern blot of mRNA from a panel of culture cell lines;

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(d) detecting differences between said genes and equivalent region of the DNA of an individual afflicted with a mood disorder or related disorder; and

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(e) identifying said gene which is associated

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with said mood disorders or related disorders.

5 If the cloned YAC DNA is sequenced, computer analysis can be used to establish the presence of relevant genes. Techniques such as homology searching and exon prediction may be applied.

10 Once a candidate gene has been isolated in accordance with the methods of the invention more detailed comparisons may be made between the gene from a normal individual and one afflicted with a mood disorder such as a bipolar spectrum disorder. For example, there are two methods, described as "mutation testing", by which a mutation or polymorphism in a DNA sequence can be identified. In the first the DNA sample may be tested for the presence or absence of one specific mutation but this requires knowledge of what the mutation might be. In the second a sample of DNA is screened for any deviation from a control (normal) DNA. This latter method is more useful for identifying candidate genes where a mutation is not identified in advance.

25 In addition, the following techniques may be further applied to a gene identified by the above-described methods to identify differences between genes from normal or healthy individuals and those afflicted with a mood disorder or related disorder:

30 (a) Southern blotting techniques: a clone is hybridized to nylon membranes containing genomic DNA digested with different restriction enzymes of patients and healthy individuals. Large differences between patients and healthy individuals can be visualized using a radioactive labelling protocol;

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(b) heteroduplex mobility in polyacrylamide gels: this technique is based on the fact that the mobility of heteroduplexes in non-denaturing polyacrylamide gels is less than the mobility of homoduplexes. It is most effective for fragments under 200 bp;

(c) single-strand conformational polymorphism analysis (SSCP or SSCA): single stranded DNA folds up to form complex structures that are stabilized by weak intramolecular bonds. The electrophoretic mobilities of these structures on non-denaturing polyacrylamide gels depends on their chain lengths and on their conformation;

(d) chemical cleavage of mismatches (CCM): a radiolabelled probe is hybridized to the test DNA, and mismatches detected by a series of chemical reactions that cleave one strand of the DNA at the site of the mismatch. This is a very sensitive method and can be applied to kilobase-length samples;

(e) enzymatic cleavage of mismatches: the assay is similar to CCM, but the cleavage is performed by certain bacteriophage enzymes.

(f) denaturing gradient gel electrophoresis: in this technique, DNA duplexes are forced to migrate through an electrophoretic gel in which there is a gradient of increasing amounts of a denaturant (chemical or temperature). Migration continues until the DNA duplexes reach a position on the gel wherein the strands melt and separate, after which the denatured DNA does not migrate much further. A single base pair difference between a normal and a mutant DNA duplex is sufficient to cause them to migrate to

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different positions in the gel;

(g) direct DNA sequencing.

5 It will be appreciated that with respect to the
methods described herein, in the step of detecting
differences between coding regions from the YAC and
the DNA of an individual afflicted with a mood
disorder or related disorder, the said individual may
10 be anybody with the disorder and not necessary a
member of family MAD31.

15 In accordance with further aspects the present
invention provides an isolated human gene and variants
thereof associated with a mood disorder or related
disorder and which is obtainable by any of the above
described methods, an isolated human protein encoded
by said gene and a cDNA encoding said protein.

20 In the experimental report which follows
reference will be made to the following figures:

FIGURE 1 shows a sequence of nucleotides which is
the left arm end-sequence of YAC 766.f.12;

25 FIGURE 2 shows a sequence of nucleotides which is
a right arm end-sequence of YAC 766.f.12;

30 FIGURE 3 shows a sequence of nucleotides which is
a left arm end-sequence of YAC 717.d.3;

FIGURE 4 shows a sequence of nucleotides which is
a right arm end-sequence of YAC 717.d.3;

35 FIGURE 5 shows a sequence of nucleotides which is

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a right arm end-sequence of YAC 731.c.7;

FIGURE 6 shows a sequence of nucleotides which is a left arm end-sequence of YAC 752.g.8;

FIGURE 7 shows a sequence of nucleotides which is a left arm end-sequence of YAC 942.c.3;

FIGURE 8 shows a sequence of nucleotides which is a right arm end-sequence of YAC 942.c.3;

FIGURE 9 shows a sequence of nucleotides which is a left arm end-sequence of YAC 961.h.9;

FIGURE 10 shows a sequence of nucleotides which is a right arm end-sequence of YAC 961.h.9;

FIGURE 11 shows a sequence of nucleotides which is a left arm end-sequence of YAC 907.e.1;

FIGURE 12 shows a pedigree of family MAD31;

FIGURE 13 shows the haplotype analysis for family MAD13. Affected individuals are represented by filled diamonds, open diamonds represent individuals who were asymptomatic at the last psychiatric evaluation. Dark gray bars represent markers for which it cannot be deduced if they are recombinant; and

FIGURE 14 shows the YAC contig map of the region of human chromosome 18 between the polymorphic markers D18560 and D18561. Black lines represent positive hits. YACs are not drawn to scale.

FIGURE 15 shows (a) a CAG repeat (in bold) and

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Sub E3

5 surrounding nucleotide sequence isolated from YAC 961_h_9. The sequence in italics is derived from End Rescue of the fragmented YAC. (b) PCR primers that can be used to determine the extent of trinucleotide repeats in the sequence.

10 FIGURE 16 shows (a) a CAG repeat (in bold) and surrounding nucleotide sequence isolated from YAC 766_f_12. The sequence in italics is derived from End Rescue of the fragmented YAC. (b) PCR primers that can be used to determine the extent of trinucleotide repeats in the sequence.

15 FIGURE 17 shows (a) a CAG repeat (in bold) and surrounding nucleotide sequence isolated from YAC 766_f_12. The sequence in italics is derived from End Rescue of the fragmented YAC. (b) PCR primers that can be used to determine the extent of trinucleotide repeats in the sequence.

20 FIGURE 18 shows (a) a CTG repeat (in bold) and surrounding nucleotide sequence isolated from YAC 907_e_1. The sequence in italics is derived from End Rescue of the fragmented YAC. (b) PCR primers that can be used to determine the extent of trinucleotide repeats in the sequence.

Experimental 1

30 (a) Family Data

35 Clinical diagnoses in MAD31, a Belgian family with a BPII proband were described in detail in De bruyn et al 1996. In that study only the 15 family members who

- 25 -

were informative for linkage analysis were selected for additional genotyping. The different clinical diagnoses in the family were as follows:

1 BPI, 2 BPII, 2UP, 4 Major depressive disorder (MDD),
5 1 SAM and 1 SAd.

The pedigree of the MAD31 family is shown in Figure 12.

(b) Genotyping of Family Members

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All short tandem repeat (STR) genetic markers are di-
or tetranucleotide repeat polymorphisms. Information
concerning the genetic markers used in this study was
obtained from several sources on the internet: Genome

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DataBase (GDB, <http://gdbwww.gdb.org/>), GenBank
(<http://www.ncbi.nlm.nih.gov/>), Cooperative Human
Linkage Center (CHLC, <http://www.chlc.org/>), Eccles
Institute of Human Genetics (EIHG,

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<http://www.genetics.utah.edu/>) and Généthon
(<http://www.genethon.fr/>). Standard PCR was performed
in a 25 µl volume containing 100 ng genomic DNA, 200
mM of each dNTP, 1.25 mM MgCl₂, 30 pmol of each
primer and 0.2 units Goldstar DNA polymerase

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(Eurogentec). One primer was end-labelled before PCR
with [gamma-³²P]ATP and T4 polynucleotide kinase. After
an initial denaturation step at 94°C for 2 min, 27
cycles were performed at 94°C for 1 min, at the

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appropriate annealing temperature for 1.5 min and
extension at 72°C for 2 min. Finally, an additional
elongation step was performed at 72°C for 5 min. PCR

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products were detected by electrophoresis on a 6%
denaturing polyacrylamide gel and by exposure to an X-
ray sensitive film. Successfully analysed STSs, STRs
and ESTs covering the refined candidate region are
fully described herein on pages 36 to 54.

- 26 -

(c) Lod score analysis.

Two-point lod scores were calculated for 3 different disease models using Fastlink 2.2. (Cottingham et al. 1993). For all models, a disease gene frequency of 1% and a phenocopy rate of 1/1000 was used. Model 1 included all patients and unaffected individuals with the latter individuals being assigned to a disease penetrance class depending on their age at examination. The 9 age-dependent penetrance classes as described by De bruyn et al (1996) were multiplied by a factor 0.7 corresponding to a reduction of the maximal penetrance of 99% to 70% for individuals older than 60 years (Ott 1991). Model 2 is similar to model 1, but patients were assigned a diagnostic stability score, calculated based on clinical data such as the number of episodes, the number of symptoms during the worst episode and history of treatment (Rice et al. 1987, De bruyn et al. 1996). Model 3 is as model 1 but includes only patients.

(d) Construction of the YAC contig - protocols

Growing of YACs and extraction of YAC DNA was done according to standard protocols (Silverman, 1995). For the construction of the YAC-contig spanning the chromosome 18q candidate region, the data of the physical map based on sequence tagged sites (STSs) (Hudson et al. 1995) was consulted on the Whitehead Institute (WI) Internet site (<http://www-genome.wi.mit.edu/>). CEPH mega-YACs were obtained from the YAC Screening Centre Leiden (YSCL, the Netherlands) and from CEPH (Paris, France). The YACs were analyzed for the presence of STSs and STRs, previously located between D18S51 and D18S61, by

- 27 -

touchdown PCR amplification. Information on the STSs/STRs was obtained from the WI, GDB, Généthron, CHLC and GenBank sites on the Internet. Thirty PCR cycles consisted of: denaturation at 94°C for 1 min, annealing (2 cycles for each temperature) starting from 65°C and decreasing to 51°C for 1.5 min and extension at 72°C for 2 min. This was followed by 10 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1.5 min and extension at 72°C for 2 min. A final extension step was performed for 10 min at 72°C. Amplified products were visualised by electrophoresis on a 1% TBE agarose gel and ethidium bromide staining.

(e) Ordering of the STR markers.

Twelve STR markers, previously located between D18S51 and D18S61, were tested for cosegregation with bipolar disease in family MAD31. The parental haplotypes were reconstructed from genotype information of the siblings in family MAD31 and minimalizing the number of possible recombinants. The result of this analysis is shown in Figure 13. The father was not informative for 3 markers, the mother was not informative for 5 markers. Haplotypes in family MAD31 suggested the following order for the STR markers analysed: cen-[S51-S68-S346]-[S55-S969-S1113-S483-S465]-[S876-S477]-S979-[S466-S817-S61]-tel. The order relative to each other of the markers between brackets could not be inferred from our haplotype data. The marker order in family MAD31 was compared with the marker order obtained using different mapping techniques and the results shown in Table 1 below.

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Table 1. Comparison of the order of the markers within the 18q candidate region for bipolar disorder, among several maps.

5	Marker*	Genetic maps		Radiation hybrid map
		Généthon	Marshfield	(Giacalone et al. 1996)
	D18S51		(-)3.4cM	(-)27.9 cR
10	D18S68	0 cM	0 cM	0 cR
	D18S346		5.3 cM	52.2 cR
	D18S55	0.1 cM	0 cM	72.5 cR
15	D18S969		0.6 cM	
	D18S1113	0.7 cM		
	D18S483	2.5 cM	3.2 cM	88 cR
20	D18S465	4.5 cM	5.3 cM	101.3 cR
	D18S876			
	D18S477	4.4 cM	5.3 cM	166.4 cR
25	D18S979		8.9 cM	
	D18S466	7.6 cM	11.1 cM	212.4 cR
	D18S61	8.4 cM	11.8 cM	249.5 cR
30	D18S817		5.3 cM	260.6 cR

* Order according to haplotyping results in family MAD31.

(-) Marker is located proximal of D18S68.

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- 29 -

D18S68, common to all 3 maps, was taken as the map anchor point, and the genetic distance in cM or cR of the other markers relative to D18S68 are given. The marker order is in good agreement with the order of the markers on the recently published chromosome 18 radiation hybrid map (Giacalone et al. (1996) Genomics 37:9-18) and the WI YAC-contig map (<http://www-genome.wi.mit.edu/>). However, a few discrepancies with other maps were observed. The only discrepancy with the Généthon genetic map is the reversed order of D18S465 and D18S477. Two discrepancies were observed with the Marshfield map (<http://www.marshmed.org/genetics/>). The present inventors mapped D18S346 above D18S55 based on maternal haplotypes, but on the Marshfield maps D18S346 is located between D18S483 and D18S979. The inventors also placed D18S817 below D18S979, but on the Marshfield map this marker is located between D18S465 and D18S477. However, the location of D18S346 and D18S817 is in agreement with the chromosome 18 radiation hybrid map of Giacalone et al. (1996). One discrepancy was also observed with the WI radiation hybrid map (<http://www-genome.wi.mit.edu/>), in which D18S68 was located below D18S465. However, the inventors as well as other maps placed this marker above D18S55.

(f) Lod score analysis and refinement of the candidate region.

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Lod score analysis gave positive results with all markers, confirming the previous observation that 18q21.33-q23 is implicated in BP disease, at least in family MAD31 (De bruyn et al. 1996). Summary statistics of the lod score analysis under all models

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1. *Staphylococcus aureus* (Staph. aureus) is a common cause of skin infections, such as abscesses, boils, and impetigo. It is also responsible for more serious conditions like pneumonia and sepsis.

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Table 2. Summary statistics of the two-point lod scores in MAD31.

Marker	Model 1			Model 2			Model 3		
	Z at $\theta=0.0$	Zmax	θ_{\max}	Z at $\theta=0.0$	Zmax	θ_{\max}	Z at $\theta=0.0$	Zmax	θ_{\max}
D18S51	-0.19	0.73	0.1	0.94	0.94	0.01	0.08	0.54	0.1
D18S68	-0.19	0.73	0.1	0.94	0.94	0.01	0.07	0.55	0.1
D18S346	-0.19	0.73	0.1	0.94	0.94	0.01	0.07	0.55	0.1
D18969	1.40	1.40	0.0	1.27	1.27	0.0	1.20	1.20	0.0
D18S1113	2.01	2.01	0.0	1.87	1.87	0.0	1.77	1.77	0.0
D18S876	2.01	2.01	0.0	1.87	1.87	0.0	1.77	1.77	0.0
D18S477	2.01	2.01	0.0	1.87	1.87	0.0	1.77	1.77	0.0
D18S979	-0.18	0.77	0.1	1.08	1.08	0.0	0.08	0.54	0.0
D18S817	-0.19	0.73	0.1	1.08	1.08	0.0	0.07	0.55	0.1
D18S61	-0.21	0.73	0.1	1.08	1.08	0.0	0.07	0.54	0.1

D18S55, D18S483, D18S465 and D18S466 were not informative.

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The highest two-point lod score (+2.01 at $\theta=0.0$) was obtained with markers D18S1113, D18S876 and D18S477 under model 1 in the absence of recombinants (table 2). In model 1, all individuals with a BP spectrum disorder are considered affected and fully contributing to the linkage analysis.

Before the fine mapping the candidate region was flanked by D18S51 and D18S61, which are separated by a genetic distance of 15.2 cM on the Marshfield map or 13.1 cM on the Généthon map. The informative recombinants with D18S51 and D18S61 were observed in 2 affected individuals (II.10 and II.11 in Fig. 13). However, since no other markers were tested within the candidate region it was not known whether these individuals actually shared a region identical-by-descent (IBD). The additional genetic mapping data now indicate that all affected individuals are sharing alleles at D18S969, D18S1113, D18S876 and D18S477 (Fig. 13, boxed haplotype). Also, alleles from markers D18S483 and D18S465 are probably IBD, but these markers were not informative in the affected parent I.1. Obligate recombinants were observed with the STR markers D18S68, D18S346, D18S979 and D18S817 (Table 2, fig. 13) Since discrepancies between different maps were observed for the locations of D18S346 and D18S817, the present inventors used D18S68 and D18S979 to redefine the candidate region for BP disease. The genetic distance between these 2 markers is 8.9 cM based on the Marshfield genetic map (<http://www.marshmed.org/genetics/>).

(g) Construction of the YAC contig.

According to the WI integrated map 56 CEPH megaYACs are located in the initial candidate region

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contained between D18S51 and D18S61 (Chumakov et al. (1995) Nature 377 Suppl., De bruyn et al. (1996)). From these YACs, those were selected that were located in the region between D18S60 and D18S61. D18S51 is not presented on the WI map, but is located close to D18S60 according to the Marshfield genetic map (<http://www.marshmed.org/genetics/>). To limit the number of potential chimaeric YACs, YACs were eliminated that were also positive for non-chromosome 18 STSS. As such, 25 YACs were selected (see Figure 14), and placed in a contig based on the technique of YAC contig mapping, i.e. sequences from sequence tagged sites (STSS), simple tandem repeats (STRs) and expressed sequenced tags (ESTs), known to map between D18S60 and D18S61, were amplified by PCR on the DNA from the YAC clones. The STS, STR and EST sequences used, are described from page 36 to 54. Positive YAC clones were assembled in a YAC contig map (Figure 14).

Three gaps remained in the YAC contig, of which one, between D18S876 and GCT3G01, was located in the refined candidate region. To close the gap between D18S876 and GCT3G01, 14 YAC clones (Table 3, on page 62) were further analysed. End fragments from YAC clones 766.f.12 (SV11R), 752.g.8 (SV31L), 942.c.3 (SV10R) were obtained and sequenced (see pages 55-61). Primers from these three sequences were selected, and DNA of each of the 14 YAC clones was amplified by PCR. As indicated in Table 3, overlaps were obtained between 7 YAC clones on the centromeric side, and two YAC clones on the telomeric site (717.d.3 and 907.e.1).

The final YAC contig is shown in Figure 14. In the figure, only the YAC clones which rendered unambiguous hits with the chromosome 18 STSS, STRs and ESTs are shown. In a few cases, weak positive signals were also obtained with some of the YAC clones, which

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likely represent false positive results. However, these signals did not influence the alignment of the YAC clones in the contig. Although, all YACs known to map in the region were tested as well as all available STSs/STRs, initially, the gap in the YAC contig was not closed. However, this was subsequently achieved by determining the end-sequences of the eight selected YACs (see below). The order of the markers provided by the YAC contig map is in complete agreement with the marker order provided by the WI map which integrates information from the genetic map, the radiation hybrid map and the STS YAC contig map (Hudson et al. 1995). Also, the YAC contig map confirms the order of the STR markers as suggested by the haplotype analysis in family MAD31. Moreover, the YAC contig map provides additional information on the relative order of the STR markers. For example, D18S55 is present in YAC 931_g_10 but not in 931_f_1 (Fig.14), separating D18S55 from its cluster [S55-S969-S1113-S483-S465] obtained by haplotype analysis in family MAD31. The centromeric location of D18S55 is defined by the STS/STR content of surrounding YACs (Fig. 14). If we combine the haplotype data and the YAC contig map the following order of STR markers is obtained: cen-[S51-S68-S346]-S55-[S969-S1113]-[S483-S465]-S876-S477-S979-S466-[S817-S61]-tel.

Out of the 25 YAC clones spanning the whole contig, seven YAC clones were selected in order to identify the minimal tiling path (Table 4). These 7 YAC clones cover the whole refined chromosome 18 region. Furthermore, YAC clones should preferably be non-chimeric, i.e. they should only contain fragments from human chromosome 18. In order to examine for the presence of chimerism, both ends of these YACs were subcloned and sequenced (pages 55 to 61). For each of

- 35 -

the sequences, primers were obtained, and DNA from a monochromosomal mapping panel was amplified by PCR using these primers. As indicated on pages 55 to 61, some of the YAC clones contained fragments from other chromosomes, apart from human chromosome 18.

Three YAC clones were then selected comprising the minimum tiling path (Table 5). These three YAC clones were stable as determined by pulsed field gel electrophoresis and their sizes correspond well to the published sizes. These YAC clones were transferred to other host yeast strains for restriction mapping, and are the subject to further subcloning.

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Description of the successfully analysed STSs, STRs and ESTs covering the refined candidate region.

Explanations:

- STS: Sequence Tagged Site
- STR: Simple Tandem Repeat
- EST: Expressed Sequence Tag

These markers are ordered from the centromere to the telomere. Only the markers that were effectively tested and that worked on the YACs are given.

List:

1. D18S60:

Database ID: AFM178XE3 (Also known as 178xe3, Z16781, D18S60)
Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs
Chromosome: Chr18

Primers:

Left = CCTGGCTCACCTGGCA
Right = TTGTAGCATCGTTGTAATGTTCC
Product Length = 157

Review complete sequence:

AGCTATCCTGGCTCACCTGGCAAAAATACAGTGTATACACACACACACAC
ACACACACACACACACACAGAGTGTNTTANTNATTCCAGCAAATAATATTA
CATATAAAAGATCTAATTGGTTCATCATGTAAATTTAGTAGGAACATTACA
ACGATGCTACAAGANTTTATCCAAACTGAGATTTCTTAGAATATCTGTT
AAAAGTAATTTTATTCAGTTAATAGAAATTCTATTGAAAACATCAAACCTTAT
AAAGCT

Genbank ID: Z16781

Description: H. sapiens (D18S60) DNA segment containing (CA) repeat;
clone

Search for GDB entry

2. WI-9222:

Database ID: UTR-03540 (Also known as G06101, D18S1033, 9222,
X63657)

Source: WICGR: Primers derived from Genbank sequences
Chromosome: Chr18

Primers:

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Left = GATCCCATAAAGCTACGAGGG
Right = GAGTCTAAAGACAAGAAAGCATTGC
Product Length = 99

Review complete sequence:

TCTTCTTACCCCTTGGGAAGAAGACTGTTTCCAAATAATTTGAACAGCTTG
CTGCTAAATGGGACCCAATTTTGGCCTATAGACACTTATGTATTGTTTTG
GAATACGTCAGATTGGACCAAGTGTCTTTCAGGAATGTGGCTGCAAGCAA
GGGGCTAGAAGTTCACCTCCTGACAGTATTATTAATACTATGCAAATATG
GAATAGGAGACCAATTTGATTTTCTAGGCTTTGTGGTAGAGAGGTGAAGG
TATGAGAATTAATAGCGTGTGAACAAAGTAAAGAACAGGATTCCAGAATG
ATCATTAAATTTGTTTCTATTTATTCTTTTTTGCCTCCCTAGAGATTAAGTC
CAGAAATGTACTTTCTGGCACATAAAGAAATCTTGAGGACTTTGTTTAAAC
CTTCATAAAAAACAATTTTCGGTTTCTCGGGTNNNNNNNNNNNNNNNNNN
NNNT
TCTTCTTTGTGTATTTTATTTCAAGATGAGTTGGACCCATTGCCAGTGAGT
CTGAATGTCACTGACAGCCCTGTGTTGTGCTCAGGACTCACTCTGCTGC
TGGTGGAAGTCAATGGCTTCTCTCTCTCTTATCCCATAAAGCTACGAG
GGGGACGGGAGAGGGCAGTGAATGGGAAGTAAAGAGATATTTCCAG
TAGGAAAAGCAATGCTTTCTGTCTTTAGACTCAAATGCTTAGGGAACGT
TTCATTTCTCATTTCATGGGGAAAGGCAGCCTCCTTAAATGTTTCTGAAG
AGCGGTAAATCTAGAAGCTTAAGAATTTACAGTTCCCTTCAATAACCATGA
TGACCTGAAGTTCACCTATCCCATTTTAGCATCTACTTGTTTTCCCATCT
CTTCCTTTCCAATTTTGCTTATACTGCTGTAATATTTTGTNNNNNNNNNN
NNNNNNNNNNNNNNNNNGACCAGCTAAAATTTTGCAGTTGACTTTTAACTT
AACTCATGAATTAATTAAGCAAATGAAAAATTAAAAAGTGTGACTTTT
CTCGGAGCATATATGTAGCTTTTAGGAAAGGCTGATGATGGTATAAAGTT
TGCTCATTAAGAAAAAAGACAAGGCTGATTTTGAAGAGAGTTGCTTTTG
AAATAAAATGATCA

Genbank ID: X63657
Description: H.sapiens fvt1 mRNA
Search for GDB entry

3. WI-7336:

Database ID: UTR-04664 (Also known as P15, G00-679-135, G06527, 7336,
U04313)
Source: WICGR: Primers derived from Genbank sequences
Chromosome: Chr18

Primers:

Left = AGACATTCTCGCTTCCCTGA
Right = AATTTTGACCCCTTATGGGC
Product Length = 332
Review complete sequence:

TAAGTGGCATAGCCCATGTTAAGTCCCTCCCTGACTTTTCTGTGGATGCCG
ATTTCTGTAACTCTGCATCCAGAGATTCATTTTCTAGATACAATAAATTG
CTAATGTTGCTGGATCAGGAAGCCGCCAGTACTTGTTCATATGTAGCCTTC

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ACACAGATAGACCNNNNNNNNNNNNCCAATTCTATCTTTTGTTTCCTTTTT
 CCCATAAGACAATGACATACGCTTTTAATGAAAAGGAATCACGTTAGAGG
 AAAAATATTTATTCATTATTTGTCAAATTGTCCGGGGTAGTTGGCAGAAAT
 ACAGTCTTCCACAAAGAAATTCCTATAAGGAAGATTGGAAGCTCTTCT
 TCCCAGCACTATGCTTTCTTCTTTGGGATAGAGAATGTTCCAGACATTC
 TCGCTTCCCTGAAAGACTGAAGAAAGTGTAGTGCATGGGACCCACGAA
 CTGCCCTGGCTCCAGTGAACTTGGGCACATGCTCAGGCTACTATAGGT
 CCAGAAGTCCTTATGTTAAGCCCTGGCAGGCAGGTGTTTATTAAATCT
 GAATTTTGGGGATTTTCAAAGATAATATTTACATACACTGTATGTTATA
 GAACTTCATGGATCAGATCTGGGGCAGCAACCTATAAATCAACACCTTAA
 TATGCTGCAACAAAATGTAGAATATTCAGACAAAATGGATACATAAAGACT
 AAGTAGCCCATAGGGGTCAAATTTGCTGCCAAATGCGTATGCCACCA
 ACTTACAAAAACACTTCGTTCCAGAGCTTTTCAGATTGTGGAATGTTGG
 ATAAGGAATTATAGACCTCTAGTAGCTGAAATGCAAGACCCCAAGAGGAA
 GTTCAGATCTTAATATAAATTCATTTTATTTGATAGCTGTCCCATCTG
 GTCATGTGGTTGGCACTAGACTGGTGGCAGGGGCTTCTAGCTGACTCG
 CACAGGGATTCTACAATAGCCGATATCAGAAATTTGTGTTGAAGGAATTT
 GTCTCTTCATCTAATATGATAGCGGGAAAAGGAGAGGAACTACTGCCTT
 TAGAAAATATAAGTAAAGTGATTAAAGTGCTCACGTTACCTTGACACATAG
 TTTTTCAGTCTATGGGTTTAGTTACTTTAGATGGCAAGCATGTAACCTATA
 TTAATAGTAATTTGTAAAGTTGGGTGGATAAGCTATCCCTGTTGCCGGTT
 CATGGATTACTTCTCTATAAAAAATATATATTTACCAAAAAATTTTGTGACA
 TTCTTCTCCCATCTCTTCTTGACATGCATTGTAATAGGTTCTTCTTGT
 TCTGAGATTCAATATTGAATTTCTCTATGCTATTGACAATAAAATATTATT
 GAACTACC

Genbank ID: G06527

Description: WICGR: Random genome wide STSs

4. WI-8145:

Database ID: EST102441 (Also known as D18S1234, G00-677-827, G06845,
 8145, T49159)

Source: WICGR: STSs derived from dbEST sequences

Chromosome: Chr18

Primers:

Left = GAAATGCACATAACATATATTTGCC

Right = TGCTCACTGCCTATTTAATGTAGC

Product Length = 134

Review complete sequence:

GTTGTTTGGANGCAGGTTTATTTATTATATACTTGCAATTGAATATAAGAT
 ACAGACATATATATGTGTTATGTATTTCTAGAAATGCACATAACATATATT
 GCCTATTGTTAATGTTTTTCCAGANATTTATTACAGAAGGGCATGGAG
 GGATACCTACTTATTCTTCATTATGAGAACAAATTAAGGCATTTATTAGAT
 AGGAAATTAACAGANCATCTGCTTCTATAACTTTATTAGCTACATTAAATA
 GGCAGTGAGCANTAATTTAAANCTCACCATATATAAANTANTAAATACC
 AAAGTAAAAG

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: left and right primer

PCR Conditions

Genbank ID: T49159

Description: yb09e07.s1 Homo sapiens cDNA clone 70692 3' similar to

gb:J02685

UniGene Cluster Description: Human mRNA for Arg-Serpin (plasminogen activator-inhibitor 2, PAI-2) Search for GDB entry

5. WI-7061:

Database ID: UTR-02902 (Also known as PAI2, G00-678-979, G06377, 7061, M18082)

Source: WICGR: Primers derived from Genbank sequences

Chromosome: Chr18

Primers:

Left = TGCTCTTCTGAACAACTTCTGC

Right = ATAGAAGGGCATGGAGGGAT

Product Length = 338

Review complete sequence:

AACTAAGCGTGCTGCTTCTGCAAAAGATTTTTGTAGATGAGCTGTGTGCC
TCAGAATTGCTATTTCAAATTGCCAAAATTTAGAGATGTTTTCTACATAT
TTCTGCTCTTCTGAACAACTTCTGCTACCCACTAAATAAAAACACAGAAAT
AATTAGACAATTGTCTATTAT AACATGACAACCCTATTAATCATTTGGTCT
TCTAAAATGGGATCATGCCCATTTAGATTTTCCTTACTATCAGTTTATTTT
TATAACATTAACTTTTACTTTGTTATTTATTATTTATATAATGGTGAGTTTT
AAATTATTGCTCACTGCCTATTTAATGTAGCTAATAAAGTTATAGAAGCAG
ATGATCTGTTAATTTCTATCTAATAAATGCCTTTAATTGTTCTCATAATGA
AGAATAAGTAGGTATCCCTCCATGCCCTTCTATAATAAATATCTGGAAAAA
ACATTAAACAATAGGCAAATATATGTTATGTGCATTTCTAGAAATACATAA
CACATATATATGTCTGTATCTTATATTCAATTGCAAGTATATAATAAATAAA
CCTGCTTCCAAACAACNNNNNNNNNNNNNNNGGAATTC

PCR Conditions

Genbank ID: G06377

Description: WICGR. Random genome wide STSs

6. D18S68:

Database ID: AFM243YB9 (Also known as 248yb9, Z17122, D18S68)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

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Primers:

Left = ATGGGAGAGGTAATACACCC

Right = ATGCTGCTGGTCTGAGG

Product Length = 285

Review complete sequence:

AAAGAGTTGGGGTTGTGAATTCCCACACCAGTCAACTATTGGCTATGGG
CTTACCATGGGAGACGTAATACACCCGGNACTTCCAACCTCACATACCAG
AGACATGGCTCTAGCACCCAATGGAAATATGCTGAATGTTGCAGGTGCA
AGACAGCAACAAAGCAGACAGAGGCACATAGACAAGGCACCAACAGTGT
CCACTATACCCTGACAGTGTGGAAAGTTGTAGATAGGATGAAGAGAAAG
AATACA
CGGTAGANACTTACTACNCAAAGTGTGANCTCAGACCAGCAGCATCTG
GCNAAATGGTGATCTATCACCTTCCAG

Genbank ID: Z17122

Description: H. sapiens (D18S68) DNA segment containing (CA) repeat;
clone7. WI-3170:

Database ID: MR3726 (Also known as D18S1037, G04207, HALd22f2, 3170)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = TGTGCTACTGATTAAGGTAAAGGC

Right = TGCTTCTTCAATTTGTAGAGTTGG

Product Length = 156

Review complete sequence

CTGAGACAAGGCAGGCAAACAACCTCTAAAAATCTACAATTGGTGATTGG
TGTGCTACTGATTAAGGTAAAGGCACAGAATTATACATCCAGGTTNCTAT
TACTTATGGCAGACTCAGGACCCAGGTTNAGAGACCACTGGCCTTAAGA
AAAAAATGGGGTTCTGATTTCTGGATAATAATCCAACCTCTACAAATTGA
AGAAGCAACATACCCTCTTTGTTA

Genbank ID: G04207

Description: WICGR: Random genome wide STSs

8. WI-5654:Database ID: MR10908 (Also known as D18S1259, G00-678-695, G05278,
5654)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

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Primers:

Left = CTTAATGAAAACAATGCCAGAGC

Right = TGCAAAATGTGGAATAATCTGG

Product Length = 149

Review complete sequence:

CTACAAAATGCATGTGGCTTTGGCTTTGAAATAGTACACCCTATCAAAGA
CTAAATTTTCTTAATGAAAACAATGCCAGAGCTTTTTTCATGATATTTGTT
TTTAGAGATGGGGAACAATCTGGACGTTGTTTCCTTATCTGGGTGGTAAT
CGAGGCTTAGCAATTTCCACAGCGTTACACAAATCCAGATTATTCCACA
TTTTGCAAATA

Genbank ID: G05278

Description: WICGR: Random genome wide STSs

9. D18S55:

Database ID: AFM122XC1 (Also known as 122xc1, Z16621, D18S55,
GC378-D18S55)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = GGGAAGTCAAATGCAAAATC

Right = AGCTTCTGAGTAATCTTATGCTGTG

Product Length = 143

Review complete sequence:

AGCTGAACATGCCTTTTCATGGAGCAGTTTCNAAATACACTTTTGGTACA
ATCTGCAGGTGGATATTTGGAGCTCAGGAGTTTGAGACCAGCCTGGGCA
ACATGGTGAATCCCGTCTCTACTAAATACAAAAATTAGCCAGGTGTG
GCGGCATGTGCCTGTAGNCCCAGGATGGATTGAGTGGGTGAGATATGG
AATAAGTGGTGGGAAGTCAAATGCAAAATCAATTGAGTTTGTCAATATTG
ATTCTCTATTCTAGCCTGGCGTGGTTTTTCCTCGTCACACACACACACAC
ACACACACACACACACACACACACACACAGCATAAGATTACTCAGA
AGCT

Genbank ID: Z16621

Description: H. sapiens (D18S55) DNA segment containing (CA) repeat;
clone10. D18S969:

Database ID: GATA-P18099 (Also known as G08003, CHLC.GATA69F01,
CHLC.GATA69F01.P18099)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = AACAAAGTGTGTATGGGGGTG

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Right = CATATTCACCCAGTTTGTTGC

Product Length = 365

Review complete sequence:

CAGGGAAATGCAAATGAAAACCACAATGAGTTATCTCCTCATACCTTTAAT
GATGGCTAATATTAACAAGAGATAACAAGTGTGTATGGGGGTGTGGAG
AAAAGAGAATGTNCGAACACTCTTGGTTGAAATATAAGTTGGTAGANCCA
TTATGCAAAACAGTATGAATCTTTATCAGTATAANATTAGGACCTNGCATA
TGATCNCAGCAATCNCACNTCTGNGNGATCNCACNCNCTATCTCTCTAT
ATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCT
ATCTGTCTGTCTATCTATCTATCTTCTATCTATCTATCTATCTTTCTAT
CTATCTATCTGTCTATCTATNCCGGAATATTTTTCAGCCATNNAATAAGG
AAGTCCTGCTATTTGCAACAACCTGGGTGAATATGGAGAACGTTATGCTA
AATGCAATATGCTAAAGACAGACACAGAAAGACAAGTATGACCTCACTTA
TATGTGGAACTGAAAAAGCCATACTCATTACAGCAAAGAGTAGAATGTT
GGTTACCAGGGGGCAAAGAGGGTAGAAATGAGGGGAGTGAGAAAATGTC
AATCAAAGTGTAAGAATGTTATAACATAAATAAATTCATAGAG

Genbank ID: G08003

Description: human STS CHLC.GATA69F01.P18099 clone GATA69F01.

11. D18S1113:

Database ID: AFM200VG9 (Also known as D18S1113, 200vg9, w2403)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = GTTGA CTCAAGTCCAAACCTG

Right = CAAAGACATTGTAGACGTTCTGTG

Product Length = 207

Review complete sequence:

AGCTGCATATAAACTATTCCATTTTACATTTTGAAGACATTTGTAGCCA
TGATACTTTGCTGTTGTCTGTGGGCCACCTCTTTTGAAGTGTGTAGTTA
ACTGTGCTCCTGTAATCTGTTGTCTGTTGA CTCAAGTCCAAACCTGTTCT
GCGTGGCATGTTTCTNCAACTTGATGTGATGCTATTTATCACTTTCTTTGA
AGTTAAGTCTCTATGTCTTTGTATTCTTTCTGTGTACCCAGGGATATGTTT
GTGCATGCACACGCATAAACACACACACACACACACACACACAGAGA
CAGAGACAGAGAACGTCTACAATGTCTTTGTGAG

12. D18S868:Database ID: GATA-D18S868 (Also known as G09150, CHLC.GATA3E12,
CHLC.GATA3E12.436, CHLC.496, D18S868)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

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Primers:

Left = AGCCAATAOCTTGTAGTAAATATCC

Right = GATTCTCCAGACAAATAATCCC

Product Length = 189

Review complete sequence:

GAGTGAGCCAATACCTTGTAGTAAATATCCATCTATCTTTGATGTATCTAT
GTATCTATCTTTGTATCTATATGTCTATGTATCTATGTATGTATCTAT
CTATCATCTATCTATCTATCATCTATCTATCTATCTATCTATCTATCT
ATCTATCTATATCCNTTTGGGATTATTTGTCTGGAGAATCCTGATTAAACAT
AGTCTGCTAACTTTTATCTGTATCTCCTATGTGTATGCTTCTCCTTCTTCC
TGTCTCTCTCTCTTTTGTCTCATTTAANCTCCTTCTGCGGNATATTG
GNAATTTTGATTGGANTCTGGACANTGTAGGAGTAAAAATT

Genbank ID: G09150

Description: human STS CHLC.GATA3E12.P6553 clone GATA3E12.

13. WI-9959:

Database ID: MR12816 (Also known as D18S1251, G00-678-524, G05488, 9959)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = TGCCAACAGCAGTCAAGC

Right = AGCACCTGCAGCAGTAATAGC

Product Length = 110

Review complete sequence:

ctgtttatttgaaaaaaaaaatctgtctccaagaagaaaagttcattctACCTGTTGCCAACAGC
AGTCAAGCGGACATGTTTAAAAATTTTTTAAAAAAGTATTTTTTTTCCAAC
GGNGTTTAATAGCCTCATTTTGGCTTTTGCTATTACTGCTGCAGGTGCTT
TNATTTTTTCTCTGCATTATAATTAC

Genbank ID: G05488

Description: WICGR: Random genome wide STSs

Search for GDB entry

14. D18S537:

Database ID: CHLC.GATA2E06.13 (Also known as CHLC.13, GATA2E06, D18S537, GATA-D18S537)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = TCCATCTATCTTTGATGTATCTATG

Right = AGTTAGCAGACTATGTTAATCAGGA

Product Length = 191

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Review complete sequence:

AAAGCTGAGTGAGCCAATACCTTG TAGTAAATATCCATCTATCTTTGATGT
ATCTATGTATCTATCTTTGTATCTATATGTCTATGTATCTATGTATGT
ATCTATCTATCATCTATCTATCTATCATCTATCTATCTATCTATCTAT
CTATCTATCTATCTATATCONTTNGGTATTATTNGTCTGGNGAATCCTGAT
TAACATAGTCTGCTAACTTNTATCTGTATCTNCTATGTGTATGCTTCTNCT
TCTTCTGTCTCTCTCTCTGCTTTGTCTCAATTNAAATCTCC

Genbank ID: G07990

Description: human STS CHLC.GATA2E06.P6006 clone GATA2E06.

Search for GDB entry

15. D18S483:

Database ID: AFM324WC9 (Also known as 324wc9, Z24399, D18S483)
Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs
Chromosome: Chr18

Primers:

Left = TTCTGCACAATTTCAATAGATTTC

Right = GAACTGAGCAAACGAGTATGA

Product Length = 214

Review complete sequence:

AGCTCTGCTGGAAGAGCAGGGCTGTTTCTGCACAATTTCAATAGATTCC
CCTACCCTGGGTTTTTCAGTAGATAGATAGATAGATAGATAGGTAGA
TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATTTT
ATATATAGTATATAAAATCTACACACACACACACACACACACACATA
TTTGCTTTTCCTTGACTATCATACTCGTTTGCTCAGTTCTTTTTTTTTTAA
ATTTTTGTTTGTAATCCAAAATGCTT

Genbank ID: Z24399

Description: H. sapiens (D18S483) DNA segment containing (CA) repeat;
clone

Search for GDB entry

16. D18S465:

Database ID: AFM260YH1 (Also known as 260yh1, Z23850, D18S465)
Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs
Chromosome: Chr18

Primers:

Left = ATATCCCCTATGGAAGTACAG

Right = AAAGTTAATTTTCAGGCACTCT

Product Length = 232

Review complete sequence:

AGCTCTGTCCCTCTAGAGAACGCTGACTAATATATCCCCTATGGAAGTA
CAGATGGTTTTNTAAAATAAATTTATCTGATTGTGATGAGATAATCATCA

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TTTTATGTTCAAGTGTCTTCTAAATTTTATTGTTATTGTTTTATACTCT
AAATGGTTTTTAAATATGCACATATGTGCATATTTACACACACACACACA
CACACACACACTCTCTTTATTTAGAAGCATTATAGATAGAGTGCCTGAAAA
TTAACTTTTAACCNAAAGAAAAGACAATAAGGAACAATAGGGAAGTTATCC
TTTGCTAAGGGTATGGAAAATATTCACATATTATTATAACANGTTAAACC
AAGTCATGCTTGANTATAATAGCT

Genbank ID: Z23850

Description: H. sapiens (D18S465) DNA segment containing (CA) repeat;
clone

Search for GDB entry

17. D18S968:

Database ID: GATA-P34272 (Also known as G10262, CHLC.GATA117C05,
CHLC.GATA117C05.P34272)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = GAAATTAACCAGACACTCCTAACC

Right = CTTAGAATTGCCTTTGCTGC

Product Length = 147

Review complete sequence:

GAATAAAAATATGAGGTATTAGAAATTACAGATAGGAAGAAATTAACCAG
ACACTCCTAACCACCGATNAGTTTAAAGAGGAGATAGATAGATAGATGAT
AGATAGATAGATAGATAGATAGATACCACTGAAAATGCAANCACAAATTA
GCAGATTATATGTGATGCAGCAAAGGCAATTCTAAGTAGATTCTAACTGC
TACATTGATAGCAGTACCCACTGACATTACCGGAAAGGATGGTATCCATA
ACCACCTACCTATATACCTCCGCAGCTGGANATTAGGNTTAAGCTTCTTN
GGGCNCCTGGCGGCCCNNTTGTGGTCCCCGGTNGGNCCCCGNNTTN
GNNTNGCTNNGNTTNCNTTGGNGNCCOCCNNTNGGTTTNNGGNNNNNT
NNNNNTNGNNNNNTTNCNNNNNNNNNTNTNNNNNCNNNNNNNNNTNNN
NNNNNNNNNNGGNNNNNGGNN

Genbank ID: G10262

Description: human STS CHLC.GATA117C05.P34272 clone GATA117C05.

18. GATA-P6051:

Database ID: GATA-P6051 (Also known as CHLC.GATA3E08,
CHLC.GATA3E08.P6051)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr13

Primers:

Left = GCAACAACCCTAATGAGTATACG

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Right = GAGTCTCACCAGGGCTTACA

Product Length = 149

Review complete sequence:

AAAGCTGTCTCCTTTTGTAAAGTGTGCTCAGAGGAATCTTTTTCAGTAAAT
AAAGTCTGCACCCAGACATCTCACTTTGTATACCACGGAGAATTTACCAT
GACTCTTCTCAGTGATAAACGTCAATATAGAATAATCAGGAGAAAAAGAG
AAATCCAGTAAAGAAATAAGTCTGTAGAAAGCAACAACCCTAATGAGTAT
ACGATATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATG
NATCTATCTATCTAACATTATATAAAATATATATTTCTCCTGTATTGGG
CCCTGTGTGTAAGCCCTGCTGAGACTCAAAAATTTGANTATTCCTNTTTN
T

Genbank ID: G09104

Description: human STS CHLC.GATA3E08.P6051 clone GATA3E08.

19. D18S875:Database ID: GATA-D18S875 (Also known as G08001, CHLC.GATA52H04,
D18S875)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = TCCTCTCATCTCGGATATGG

Right = AAGGCTTTCAGACTTAACTGG

Product Length = 394

Review complete sequence:

TTATTTATTCATCTCATTCAATAAATATTTATGAATTTCTTTAATGGCNANG
AAAGTATGTTTGGTACTGAATATGGTGAGCAAGATTTTCCTCTCATCTCG
GATATGGAAAGATCTTGAAATCATTATACNTCATACTTACAATANGAAAG
AAGCTGAGCAATTTGAAAATCAACAATTTCTTTGTACNTGTCAGAAAAGT
GAAGATATATTAATCAGGGTTCTTCAGAGAAACATAACCAATAGGNCACA
GNTCTATATGNCCNCNTTTATCTATCTATCTATCTATCTATCNCATCTAT
CNCANACCNGGNGAANTNATNTTTGNGAGATTNATGCAAGNCTGAGAAA
NACCNAAGAANCTGCTCCCTGTNAACTNGAGATNCAAGAANCTGAANA
GTATAGNTCCAGTCCNAAGTCTANAGACCTTAGAATTAGGAAAAGTCTGATA
CTATAAATACCAGTGTAAGTCTGAAAGCCTTAAANACCANATAGTGCCAT
TGAAAGGGCAGAAGACTGATGTCCCAGTTCAAGCAGGCAAAGTTAGAGA
AGCCTTATTTCTGCAACATTGTTCTATTCAGACCCTTNANANGATTGACN
ATGTCCACCCA

Genbank ID: G08001

Description: human STS CHLC.GATA52H04.P16177 clone GATA52H04.

Search for GDB entry

20. WI-2620:

Database ID: MR1436 (Also known as G03602, D18S890, HHAa12h3, 2620)

Source: WICGR. Random genome wide STSs

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Chromosome: Chr18

Primers:

Left = TCTCCAAGCTATTGATTGGATAA

Right = TTAAGAGCCAATTTATATAAAAGCAGC

Product Length = 177

Review complete sequence:

CCCCTTTTGCCAACGCCATGCTTCACGTAGGGAGCCTGACATGCAGAAA
ACTCTCCAAGCTATTGATTGGATAAAGAGCCAGAGCTGACTGAATTCCAT
TCTTCTTGAGCCTCTCATTCTGTGTTTCTCGAATTTTACCAAAGCATCTT
GACACACAAATATCTGACTCAAGGAAAAGGAAAAACAACCTGCTTTTTCTC
CAGCTGCTTTTATATAAATTGGCTCTTAACTTTCTAAGTTTATTATGGAT
A

Genbank ID: G03602

Description: WICGR: Random genome wide STSs

Search for GDB entry

21. WI-4211:

Database ID: MR6638 (Also known as G03617, D18S980, 4211)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = ATGCTTCAGGATGACGTAATACA

Right = AAATTCTCGCTGATTGGAGG

Product Length = 113

Review complete sequence:

CTAGTACCATAATCCCTTTTGGGAATAAACCATCCCACCTTTAGTCAGANC
AGATGCTTCAGGATGACGTAATACATAATAAGCCTACTCAGTTCTACTCT
GGCTTTGTATGTCTTCAAAGTGATATTTTTTAAGTATTACTTGTCCCTCC
AATCAGCGAGAATTI

Genbank ID: G03617

Description: WICGR: Random genome wide STSs

Search for GDB entry

22. D18S876:Database ID: GATA-D18S876 (Also known as G09963, CHLC.GATA61E10,
D18S876)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = TCAAACCTTATAACTGCAGAGAAAG

Right = ATGGTAAACCCTCCCCATTA

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Product Length = 171

Review complete sequence:

AAGACTGCAATTACATTTGCATCAAACCTTATAACTGCAGAGAACGTTGCC
CACTATTTTATACCACACAACAGTATTCTTAGCCAGATTACATCTATCTAT
CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAGC
TAGCTATCTATCTATAGAACTAATGGGGAGGGTTTACCATGTTTGGGTGA
ACCCAAACATTTTATGGNCAAGGGNTTGGAAAATTACCCTTATCTACAAC
TNTTNAACCTTGTTTTGGTAGGNGTGNTAATTCNTGGGNTTGAANAAC
TTTGNAATTCCTCNTTGTNTNATTNNNNATTNNTNNNCATTATTNTGG
GGTNTTCNGGGTGGAGGGCTNANTTTGGCCNCCCGGGTCCNNGGNGC
NAGTNGGNNNGGNTNNTNGGGTTTNCCTTGGAANCNTNCCNCCTNCNG
GGGNTTCANGGGNTTTTNTTTNTTTG

Genbank ID: G09963

Description: human STS CHLC.GATA61E10.P17745 clone GATA61E10.

Search for GDB entry

23. GCT3G01:

Database ID: GCT-P10825 (Also known as G09484, CHLC.GCT3G01,
CHLC.GCT3G01.P10825)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats
Chromosome: Chr18

Primers:

Left = CTTTGCAATCTTAGTTAATTGGC

Right = GAACTATGATATGGAGTAACAGCG

Product Length = 128

Review complete sequence:

AGATGTTTAACTTTGCAATCTTAGTTAATTGGCAGAAATGAAATTTAGTTT
CCACAACCTTTTATTCGATATTAACACACCACCACCATCAGCAGCAGCAGC
AGCAGCAGCAGCATCGCTGTTACTCCATATCATAGTTTACAGAGCATTAAA
GNGGTCAAAATATACTAGGCTGACACCNAGNATAAGGTTTAATTTTAA
ACCNNGGTCCTNCCCTCTAAGGNGGNTTTTTTTTCTTGNCNTGGCTTCT
TTTTCCNTTTGCTTTGTAAAATATCAAGGNATTTTGGGTTNTTCNTGGN
ANTTNNCANNANTNNTNNTTNNNCNCCCCCCTTTGNGGCGGGGGTC
CCCNNTTGCCCCGGGGTTGNGTGACGTAGGGGGGTCNCGGGTNNNG
NAAGTTTNGGGGCCCT

Genbank ID: G09484

Description: human STS CHLC.GCT3G01.P10825 clone GCT3G01.

24. WI-528:

Database ID: MH232 (Also known as G03589, 528, D18S828)

Source: WICGR: Random genome wide STSs

Chromosome: Chr13

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Primers:

Left = TTCTGCCTTTCCTGACTGTC

Right = TGTTCCTCATGTCTTGATGA

Product Length = 211

Review complete sequence:

CTACTAAGCAAATTCTGCTCAGCCTTCTGCCTTTCCTGACTGCTTGTTG
GCCCTTCCCACCTTAAAGGATGCCTGTTTAAGTAGCCACCTCTAATTAGGA
ATCTTCCCTTGTTCTTCTCAGGAGGCTTAGACACTGTCAGTTTCCTGAA
GACAGAAAATAAGCCTGCATTATCCTAGTAGTGGAATTCAAACTAATTGT
GTCCTGAGTCTTTCATCATCAAGACATGGGAAACACTCAACAG

Genbank ID: G03589

Description: WICGR: Random genome wide STSs

Search for GDB entry

25. WI-1783:

Database ID: MR432 (Also known as G03587, _shu_31.Seq, 1783,
D18S824)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = CCAGTAATTAGACATTGACAGGTTTC

Right = TTTTACTAGACAGGOTTGATAAACAA

Product Length = 305

Review complete sequence:

CCAGTAATTAGACATTGACAGGTTCCATACTAGTAATGTAGGGAATAGGG
CTGCTGCTTTTTGGGTTTCCTTGAGTATACTTTGTGCTGCATAAATATGG
CAATGGATAGTAAATAATTTGTATGCAGACCTTTAGTGTCGATTAACTGT
GAATAAGGGAACAACAATCAAGGACAAAAATCAAAAGACTAATTCTCTAT
ACATTTTGAGCTTTTGTAAGAAAGTAAGATTAGCTGAATATATCTGAAAAA
TTTCTAATCTCCTTTACAATTTTTAAATTGTTTATCAAGCCTGTCTAGTAA
AAATAATTCAGTTTCGGAATGTGG

Genbank ID: G03587

Description: WICGR: Random genome wide STSs

Search for GDB entry

26. D18S477:

Database ID: AFM301XF5 (Also known as B01xf5, Z24212, D18S477)
Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs
Chromosome: Chr18

Primers:

Left = GGACATCCTTGATTTGCTCATAA

Right = GATTGACTGAAACAGGCACAT

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Product Length = 243

Review complete sequence:

Review complete sequence:
 GGACATCCTTGATTGCTCATAATACACTCATTCCCTTTCACCATTGAGTGT
 GCACATATTTCTCTGATTGGAAAGAACTACAGAGGAGGTTTTACNTTTTA
 CTTTCCAGTTTGCTATTAAGAGAGAAAACTAACAGAGNGAAATCAAGCA
 ACTCAAAACAACCTTACACACACACACACACACACTCACAAAGATA
 TTTTGTTCACCATATGATTGATGTGCCTGTTTTCAGTCAATCCACAGGAA
 GGGCTAAGGAGAGTGACATCTGGGCTACATTAAAAGGACAGTCACATTG
 CTCAAAGNACTCAAGTTTAGCCCGAGTACAGTAGCT

Genbank ID: Z24212

Genbank ID: Z24212
Description: H. sapiens (D18S477) DNA segment containing (CA) repeat;
clone

Search for GDB entry

27. D18S979:

Database ID: GATA-P28080 (Also known as G08015, CHLC.GATA92C08,
CHLC.GATA92C08.P28080)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats
Chromosome: Chr18

Primers:

Left = AGCTTGCAGATAGCCTGCTA

Right = TACGGTAGGTAGGTAGATAGATTCTG

Product Length = 155

Review complete sequence:

[illegible]

Genbank ID: G08015

Genbank ID: G08015
Description: human STS CHLC.GATA92C08.P28080 clone GATA92C08.

28. WI-9340:

Database ID: UTR-05134 (Also known as G06102, D18S1034, 9340, X60221)

Source: WICGR: Primers derived from Genbank sequences
Chromosome: Chr18

Primers:

Left = TGAGAGAACGAAATCTCTATCGG

Right = AGGCAGCAAGTTTTTATAAAGGC

Product Length = 115.

Review complete sequence:

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ATGTATCTATCCCAATTGAGTCAGCTAGAAACAGTTGACTGACTAAATGG
AAACTAGTCTATTTGACAAAGTCTTTCTGTGTTGGTGTCTACTGAAGTTAT
AGTTTACCCTTCCTAAAAATGAAAAGTTTGTTCATATAGTGAGAGAACGA
AATCTCTATCGGCCAGTCAGATGTTTCTCATCCTTCTTGCTCTGCCTTTG
AGTTGTTCCGTGATCATTCTGAATAAGCATTGCTTTATAAAAACTTGCT
GCCTGACTAAAGATTAACAGGTTATAGTTTAAATTTGTAATTAATTCTACC
ATCTTGCAATAAAGTGACAAATTGAATG

Genbank ID: G06102

Description: WICGR: Random genome wide STSs

Search for GDB entry

29. D18S466:

Database ID: AFM094YE5 (Also known as 094ye5, Z23354, D18S466)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = ACACTGTAGCAGAGGCTTGACC

Right = AGGCCAAGTTATGTGCCACC

Product Length = 214

Review complete sequence:

aaatgacactttaaggaggtaacactgtacagagccttgaccaccaccagttctactagcactgagg
atgctctattgggtgggttaccacacacgcataagacatgcacacacagacacacagacacacac
acacacacacacaccagatatagcattccaaaccatcaatatgctatgcaatactgcattaacaggcatg
cctgaggtggcacataactggcctagaaaatactggggacgtctgattccctttattatcgaattgacttact
tggcttctgagtttctcagaagtaataacttcaatacctctccattctgccttgancattgttgggtaccaag
tatagct

Genbank ID: Z23354

Description: H. sapiens (D18S466) DNA segment containing (CA) repeat;
clone

Search for GDB entry

30. D18S1092:

Database ID: AFMA112WE9 (Also known as D18S1092, w5374, a112we9)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = CTCTCAAAGTAAGAGCGATGTTGTA

Right = CCGAAGTAGAAAATCTTGCA

Product Length = 163

Review complete sequence:

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Markers (STRs) used in refining the candidate region.

Below the markers are shown that were used in family MAD31 to refine the candidate region. Most of these markers are already described above and will therefore only be mentioned to by their name. For the additional markers, the information is given here.

Data was already shown for: D18S68, D18S55, D18S969, D18S1113, D18S483, D18S465, D18S876, D18S477, D18S979, D18S466 and D18S61.

New data:

1. D18S51:

Other names: UT574, (D18S379)

Primer sequences:

UT574a GAGCCATGTTGATGCCACTG
UT574b CAAACCCGACTACCAGCAAC

DNA-sequence:

AATTGAGCNCAGGAGTTTAAGACCAGCCTGGGTAACACAGTGAGACCCC
TGTCTCTACAAAAAATACAAAAATNAGTTGGGCATGGTGGCACGTGCCT
GTAGTCTCAGCTACTTGCAGGGCTGAGGCAGGAGGAGTTCTTGAGCCCA
GAAGGTTAAGGCTGCAGTGAGCCATGTTGATGCCACTGCACTTCACTCT
GAGTGACAAATTGAGACCTTGTCTCAGAAAGAAAGAAAGAAAGAAAGAA
GAAAGAAAGAAAGAANGAAAGAAAGAAAGTAAGAAAAAGAGAGGGAAAG
AAAGAGAAANAGNAAAATAATAGTAGCAACTGTTATTGTAAGACATCTCC
ACACACCAGAGAAGTTAATTTTAATTTAACATGTTAAGAACAGAGAGAAG
CCAACATGTCCACCTTAGGCTGACGGTTTGTGTTATTTGTGTTGTTGCTGG
TAGTCGGGTTTGTGTTATTTTAAAGTAGCTTATCCAATACTTCATTAACAAT
TTCAGTAAGTTATTTTATCTTTCAACATAAATACGNACAAGGATTTCTTCT
GGTCAAGACCAAACTAATATTAGTCCATAGTAGGAGCTAATACTATCACA
TTTACTAAGTATTCTATTTGCAATTTGACTGTAGCCCATAGCCTTTTGTGCG
GCTAAAGTGAGCTTAATGCTGATCGACTCTAGAG

GENBANK ID: L18333

2. D18S346:

Other name: UT575

Primer Pairs:

Primer A: TGGAGGTTGCAATGAGCTG
Primer B: CATGCACACCTAATTGGCG

DNA sequence:

ACGAGGACAGGAGTTCAAGACCAGCCTGGCCAACATGGTGAACCCCGTT
TNTACTAAAANTACAAAANTTGGTCGGGAGGCTGGGGCAGGNGACATGC

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Characterisation of YACs.

8 YACs were selected covering the candidate region and flanking the gap. These YACs were further characterised by determining the end-sequences by the Inverse-PCR protocol.

Selected YACs: 961_h_9, 942_c_3, 766_f_12, 731_c_7, 907_e_1, 752_g_8, 717_d_3, 745_d_2

New STSs based on end-sequences (unless indicated otherwise, the STSs were tested on a monochromosomal mapping pannel for identifying chimaerism of the YAC; if the STS revealed a hit not on chromosome 18q - chimaeric YAC- then it is indicated in the text below):

1. SV32L.

Derived from YAC 745_d_2 left arm end-sequence.

Primer A: GTTATTACAATGTCACCCCTCATT
Primer B: ACATCTGTAAGAGCTTCACAAACA

DNA-sequence:

ATTCCTTNGTTATTACAATGTCACCCCTCATTTAAAAAGTGGAAAGATAAAG
AGGAAGCAATCTATTTTTTCTTTTCTGATAGCACTTGTTTGTGAAG
CTCTTACAGATGTTCTTAAGTAAAATCAACTCCTCCATTTTTTTGTAGCA
ACTACACATATTTATCAATAATAGTTCACAAATACATTTTCAAATT

Amplified sequence length: 107 basepairs (bp)

This STS has no clear hit on the monochromosomal mapping pannel.

2. SV32R.

Derived from YAC 745_d_2 right arm end-sequence.

Primer A: ACGTTTCTCAATTGTTTAGTC
Primer B: TGTCTTGGCATTATTTTAC

DNA sequence:

AGACAATGGGAGAAATTGCACTGCCCTGAGTCAGAAATCAGATCTGTTG
CCATACAGCTGCCGTTATGTGATCATTTGCAAGTCAACGTTTCTCAATTG
TTTAGTCATTTGTAAGACAAAAAGACTGGTTGGATTTCAGAGAATTTGGA
ATCCTCCTTCAGGTTTAACAAGCAATAAATGATACTCTTCAGTGTAAAAAT
AATGCCAAGACATNATTTGACTTTAAATTAATCCAAACAAGATATC

Amplified sequence length: 127 bp

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This STS has no clear hit on the monochromosomal mapping pannel.

3. SV11L

Derived from YAC 766_f_12 left arm end-sequence.

Primer A: CTATGCTCTGATCTTTGTTACTTT
Primer B: ATTAACGGGAAGAATGGTAT

DNA sequence:

GTCTTTATTTTCATATAACTATGCTCTGATCTTTGTTACTTTCTCCTTTTAAC
TCAGTTTAAGCTTTTATCTTATTTTCCAGCTGCTGAAGGTATATAGTTAGG
TTGTTTATTGGATACCATTCTTTCCCGTTAATGTCAGTGGTACTGCTATC
AATGTAGCAGTTA

Amplified sequence length: 118 bp

This STS has a hit with chromosome 18 and must be located between
CHLC.GATA-p6051 and D18S968.

4. SV11R

Derived from YAC 766_f_12 right arm end-sequence.

Primer A: AAGGTATATTATTTGTGTCG
Primer B: AAACCTTTCTTAACCTCATA

DNA sequence:

ATAAGGTATATTATTTGTGTCGTGAGTTAAGAAATCATTAACTATTTT
CAGAATGACAAATGTCATTATATGTTGTAAAAAGATAAATACGTGAAAT
ATGAGGTTAAGAAAAGTTTA

Amplified sequence length: 119 bp.

This STS has a hit with chromosome 18 and must be located between
D18S876 and GCT3G01.

5. SV34L

Derived from YAC 717_d_3 left arm end-sequence.

Primer A: TCTACACATATGGGAAAGCAGGAA
Primer B: GCTGGTGGTTTTGGAGGTAGG

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DNA sequence:

ACATAAAATGTCGCTCAAAAACAATTATGTGTGTCTACACATATGGGAAA
GCAGGAAACAAATTTGTTACAACATACATTACTTTTGTITTTTAGGCAAG
ATAAAATNTCCTACCTCCAAAACCACCAGCACNGTCCGCAATAACTATAC
ATC

Amplified sequence length: 98 bp

This STS has a hit with chromosome 18.

6. SV34R.

Derived from YAC 717_d_3 right arm end-sequence.

Primer A: ATAAGAGACCAGAATGTGATA

Primer B: TCTTTGGAGGAGGGTAGTC

DNA-sequence:

AATATCATTCTTCACCCACGTTATACATAAGAGACCAGAATGTGATATTGT
CATCTCACATGGAAAAATCTGCTGTGATCAGTTCCTGAAGCTTGCTGTGA
TCCTCCCTTAGGAAAGTAGAAAAATCTTTTGAACACTTTATTCTACAAT
CAATGAAAATTAGGTGAAGCTACAGAAGCCAGAAATTACTCTAAGATTAG
ACAATTATTTAAGANGACCAATTGTCCTTGGTCTTCTTCTGAAGGGTCTG
ACTACCCTCCTCCAAAGAATTCACTGGCCGTCGTTTTACAACGTONTGA

Amplified sequence length: 244 bp

This STS has a hit with chromosome 1, therefore YAC 717_d_3 is chimaeric

7. SV25L

Derived from YAC 731_c_7 left arm end-sequence.

Primer A: AAATCTCTTAAGCTCATGCTAGTG

Primer B: CCTGCCTACCAGCCTGTC

DNA sequence:

AGTGGAGAGATAGAAAGAGAGGAAGATTTTITTTTTAAATCTCTTAAGCT
CATGCTAGTGTAGGTGCTGGCAGGTCTGAACACTCTGTAGGACAGGCTG
GTAGGCAGGAA

Amplified sequence length: 72 bp

This STS has no clear hits on the monochromosomal mapping pannel.

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8. SV25R.

Derived from YAC 731_c_7 right arm end-sequence.

Primer A: TGGGGTGCGCTGTGTTGT

Primer B: GAGATTCATGCATTCCTGTAAGA

DNA-sequence:

GGAGGGTGTTNTCACANAAGTCTGGGGTGCGCTGTGTTGTTTCATTGTAA
AAACCCTTTGGANCATCTGGAATGTGCTGCCCCACATGTCCAGGTAAC
GTTCTCAGGAAGGGGAGGCTGGAAATCTCTGTGTGTTCTTACAGGAATG
CATGAAATCTCCCANCCCCCTCTTGTTGGAAATTCCTCACTTT

Amplified sequence length: 136 bp

This STS has a hit with chromosome 7; therefore YAC 731_c_7 is chimaeric

9. SV31L.

Derived from YAC 752_g_8 left arm end-sequence.

Primer A: GAGGCACAGCTTACCAAGTTCA

Primer B: ATTCATTTTCTCATTTTATCC

DNA-sequence:

CTTCTCNATGANTGGACAAATGTCATTGGGTCAGCATGAGGCACAGCTT
ACCAAGTTTCAGATTCCAGTAGCTGAGGAACAAATCTTAACTCCAAAAATAA
GTAATTGCGTCACCTTTGGAGGAATTTATTTGACCTTTTCATAACTTTGACAT
CACAAATGAGGGTGAAGTTAGTAAAATAAATGATTATTATGAGGATAA
AATGAGAAAATGAATTNAGTGCTTAAGACAATGCTTGGTAACTAGTTAAN
CCG

Amplified sequence length: 178 bp

This STS has a hit with chromosome 18 and must be located between
D18S876 and GCT3G01.10. SV31R.

Derived from YAC 752_g_8 right arm end-sequence.

Primer A: CAAGATTATGCCTCAACT

Primer B: TAAGCTCATAATCTCTGGA

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DNA sequence:

AAACTTTAACCAATTTAAACTCCCTAACAGTTCTATAAAATAAGCAAGATT
 ATGCCTCAACTTTATGGATAAAGAAATGGAGGCATTAAGAGATAACTAAC
 TTGCCCAAGGCCACACAAGTGACTGAGTAAGAATTGCAAAGCCAATGAG
 TCTGGCTCCAGAGATTATGAGCTTAATCACCACACTGTGCCACCTCCTGT
 GTTTCCTGG

Amplified sequence length: 131 bp

This STS has no clear hits on the monochromosomal mapping pannel and
 gives no information concerning the chimaerity of the YAC.

11. SV10L.

Derived from YAC 942_c_3 left arm end-sequence.

Primer A: TCACTTGGTTGGTTAACATTACT
 Primer B: TAGAAAAACAGTTGCATTGATAT

DNA-sequence:

GGTNTTTCACCTTGGTTGGTTAACATTACTTCTAAGTTTTTATTGTTTTTA
 TGCTATTGCTAATGGGATTGCTTTCTTAATTTATTTTCCAATAGCTTGT
 TGTTAGTTTATATCAAATGCAACTGTTTTCTATGCAAATTATGTTTCCT

Amplified sequence length: 130 bp

This STS has a hit with chromosome 18 and must be located between
 CHLC.GATA-p6051 and D18S968

12. SV10R.

Derived from YAC 942_c_3 right arm end-sequence.

Primer A: AACCCAAGGGAGCACAACTG
 Primer B: GGCAATAGGCTTTCCAACAT

DNA sequence:

TTGGTGGTGCCCTAGGTTTGGCAATTATAAATAAAGCTGCTACAAACATT
 CATGTGCAGGTCTCCGTGTGGACATAATTTCCAGTTCATTTGGGTAAAA
 CCCAAGGGAGCACAACTGTTGGATCCTATNATAAAAAATATNTCTCGTTTC
 ATTTAAAAAACCTGGGAACTATCTNCCACAGTGGCTGTCCCTTTTGT
 ATCCCACCAACAATGTTGGAAAGCCTATTGCCANCAT

Amplified sequence length: 135 bp

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This STS has a hit with chromosome 18 and must be located between D18S876 and GCT3G01

13. SV6L

Derived from YAC 961_h_9 left arm end-sequence.

No primer was made, because this sequence is identical to a known STR marker D18S42, which is indeed mapped to this region.

Primer A:

Primer B:

DNA sequence:

CATGNCTCACAGTGTCTGAGGCTGCTCTGGACATGCAATCTTGCATGC
TTTTGTCATGACAGGTCTTAAANAGTTTATCAGCTTNTCAAATAGCTGAA
TGACANAACACTGGATTTTGTTCAAATANCCTATCAACTTGGCNTCTGT
GTTGCGGTTGTCACTTGGTAACAAAATAAGTC

Amplified sequence length:

SV6L recognises D18S42 which must be therefore located between WI-7336 and WI-8145

14. SV6R

Derived from YAC 961_h_9 right arm end-sequence.

Primer A: TTGTGGAATGGCTAAGT

Primer B: GAAAGTATCAAGGCACTG

DNA sequence:

TAATTGACAAATAAAAATTGTATATTTTNCATATTTAACATGTTATGCTAAC
ATATATATGGATTGTGGAATGGCTAAGTCAGAAATCTTTTACATTCATAT
TTCCATATTATTTACTTTNNGCTTTAAAAAATATGTAAATGANAATACTTAT
TTTTTTCAGTGTCCTTGCCTTGATACTTTCACATTTNNGTTACATATTATTT
CCCTTNCATCTAACAAATATATATTGAGTTTCTATAATGTGTCTGACACTG
A

Amplified sequence length: 122 bp

SV6R amplifies a segment on chromosome 18. This segment must be located between WI-2620 and WI-4211

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15. SV26L.

Derived from YAC 907_e_1 left arm end-sequence.

Primer A: TATTTGGTTTGTTTGCTGAGGT
Primer B: CAAGAAGGATGGATACAAACAAG

DNA sequence:

TGGTCACTGGTGCCTTATTTGGTTTGTTTGCTGAGGTCATATTCCTGTG
GCCTTCATGCTTGATTTGTTGGAGTCTAGCCATGTAAAANTCTGTTGGAG
TCTAGGCATTTAAAAATAGGTATTTATTGTAATCTTTGCCATTGCTTGT
TTGTATCCATCCTTCTTGGAAGGCTTTACAGGCATTCAAAGG

Amplified sequence length: 154 bp

This STS has a hit with chromosome 13; therefore YAC 907_e_1 is chimaeric.

16. SV26R.

Derived from YAC 907_e_1 right arm end-sequence.

Primer A: CGCTATGCATGGATTTA
Primer B: GCTGAATTTAGGATGTAA

DNA sequence:

CGCTATGCATGGATTAACTGAGTGTAGTGCCTCACTATGTTGCAGTC
TCTTATTCTAGGTTCTAATAATTACATCCTAAATTCAGCT

Amplified sequence length: 90 bp

no clear hits on monochromosomal mapping pannel: no information concerning chaemerity at this side of the YAC

SubE4

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

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Testing of 3 end-sequences flanking the gap in additional YACs: STS-markers WI-4211, D18S876 and GCT3G01 are also shown in order to identify YACs on opposite sides of the gap more clearly in table 3 below.

5

YACs	STSs					
	WI-4211	D18S876	SV31L	SV11R	SV10R	GCT3G01
940_b_1	+	+	+	-	-	-
766_f_12	+	+	+	+	-	-
846_a_5	+	- ?	+	+	-	-
752_g_8	+	+	+	+	-	-
745_d_2	+	+	+	+	-	-
961_c_1	+	+	-	-	-	-
942_c_3	+	+	+	+	+	-
717_d_3	-	-	+	+	- ?	+
972_e_11	-	-	-	-	-	+
940_h_10	-	-	-	-	+	+
821_e_7	-	-	-	-	+	+
731_c_7	-	-	-	-	-	+
889_c_4	-	-	-	-	+	+
907_e_1	-	-	-	+	+	+

20

- +: positive hit / -: no hit / ?: 2 instances were observed in which a positive hit was expected (on the assumed order of the markers) but not observed. The reasons for this are not clear.

25

YAC 745_d_2 was excluded from further analysis since there was no clear link with chromosome 18. Of the remaining 7 from a monochromosomal mapping panel it was determined that 3 were chimeric and 4 non-chimeric as shown in Table 4 below.

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TABLE 4

	YAC	chimaeric	chromosome
s	961_h_9 (6)	no	
	942_c_3 (10)	no	
	766_f_12 (11)	no	
	731_c_7 (25)	yes	chromosome 7
	907_e_1 (26)	yes	chromosome 13
10	752_g_8 (31)	no	
	717_d_3 (34)	yes	chromosome 1

For the non-chimeric YACs the STS based on the end-sequence flanking the gap (10R, 11R, 31L) was tested on 14 YACs flanking the gap. Overlaps between YACs on opposite sides of the gap were demonstrated: e.g. the "11R" end-sequence (766.f.12) detects YAC 766.f.12 and YAC 907.e.1.

YACs were then selected comprising the minimum tiling path:

TABLE 5

	YAC	size	chimaerity
25	961_h_9	1180 kb	not chimaeric
	766_f_12	1620 kb	not chimaeric
	907_e_1	1690 kb	chimaeric (chr. 13)

These three YACs are stable as determined by PFGE and their sizes roughly correspond to the published sizes. These YACs were transferred to other host-yeast strains for restriction mapping.

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Experimental 2Construction of fragmentation vector:

SubES

5 A 4.5kb ECORI/SalI fragment of pBLC8.1 (Lewis et al, 1992) carrying a lysine-2 and a telomere sequence was directionally cloned into GEM3zf(-) digested with ECORI/SalI. Subsequently, an End Rescue Site was ligated into the EcoRI site. Hereto, two

10 oligonucleotides (strand 1: 5'-TTCGGATCCGGTACCATCGAT-3' AND STRAND 2: 3'-GCCTAGGCCATGGTAGCTATT-5') were ligated into a partial (dATP) filled ECORI site, generating the vector pDF1. Triplet repeat containing fragmentation vectors were constructed by cloning of a

15 21bp and a 30bp CAG/CTG adapter into the Klenow-filled PstI site of pDF1. Trasformation and selection resulted in a (CAG)₇ and a (CTG)₁₀ fragmentation vector with the orientation of the repeat sequence 5' to 3' relative to the telomere.

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Yeast transformation:

Linearised (digested with SalI) vector was used to transform YAC clones 961.h.9, 766.f.12 or 907.e.1

25 using the LiAc method. After transformation the YAC clones were plated onto SDLys⁻ plates to select for the presence of the fragmentatio vector. After 2-3 days colonies were replica plated onto SDLys⁻-Trp⁻-Ura⁻ and SDLys⁻-Trp⁻-Ura⁺ plates. Colonies growing on the

30 SDLys⁻-Trp⁻-Ura⁺ plates but not on the SDLys⁻-Trp⁻-Ura⁻ plates contained the fragmented YACs.

Analysis of fragmented YACs:

35 Yeast DNA isolated from clones with the correct

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phenotype was analysed by Pulsed Field Electrophoresis (PFGE), followed by blotting and hybridisation with the Lys-2 gene and the sizes of the fragmented YACs were estimated by comparison with DNA standards of known length.

End Rescue:

Fragmented YACs characterised by a size common to other fragmented YACs, indicative of the presence of a major CAG or CTG triplet repeat, were digested with one of the enzymes from the End Rescue site, ligated and used to transform E. Coli. After growth of the transformed bacteria the plasmid DNA was isolated and the ends of the fragmented YACs, corresponding to one of the sequences flanking the isolated trinucleotide repeats, were sequenced.

Sequencing revealed that fragmented YACs of an equal length were all fragmented at the same site. A BLAST Search of the GenBank database was performed with the identified sequences to identify homology with known sequences. The complete sequence spanning the CAG or CTG repeats of the fragmented YACs was obtained by Cosmid Sequencing, employing sequence specific primers and splice primers, as previously described (Fuentes et al. 1992 Hum.Genet. 101: 346-350) or by using the "genome walker" kit (Clontech Laboratories, Palo Alto, USA) and described in Siebert et al. Nucleic Acid Res (1995) 23(6): 1087-1088 and Siebert et al. (1995) CLONTECHniques X(II): 1-3.

Results:

A YAC 961.h.9 clone was transformed with the (CAG)₇ or (CTG)₁₀ fragmentation vector. The CTG vector

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did not reveal the presence of any CTG repeat.
Analysis of twelve (CAG)₇ fragmented YACs showed that
five of these had the same size of approximately
100kb. End Rescue was performed with ECORI and
sequencing of three of these fragments revealed that
they all shared the terminal sequence shown in italics
in Figure 15a. A BLAST search of the Genbank database
with this sequence indicated the presence of a
sequence homology with the CAP2 gene (GenbBank
accession number: L40377). The sequence spanning the
CAG repeat shown in Figure 15a was obtained by both
cosmid sequencing and genome walker sequencing. The
sequence was mapped between markers D18S63 and WI-3170
by STS content mapping.

A YAC 766-f-12 was fragmented using the
(CAG)₇ or (CTG)₁₀ fragmentation vector. Again the
(CTG)₁₀ vector did not reveal the presence of any CTG
repeat. Analysis of twenty (CAG)₇ fragmented YACs
showed the presence of two groups of fragments with
the same size: five of approximately 650kb and two
of approximately 50kb.

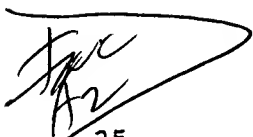
End Rescue was performed using ECORI on four of
the fragmented YACs of 650kb. Sequencing confirmed
that they all shared identical 3' terminals,
characterised by the sequence shown in italics in
Figure 16a. A Blast Search showed homology of this
sequence with the Alu repeat sequence family. The
sequence spanning the CAG repeat shown in Figure 16a
was obtained by cosmid sequencing. The sequence was
mapped between markers WI-2620 and WI-4211 by STS
content mapping on the YAC contig map.

End Rescue was also performed on the two fragments of
50kb. Sequencing revealed the sequence shown in
italics in figure 17a. A Blast Search revealed no

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sequence homology with any known sequence. Cosmid sequencing allowed to identify the complete sequence spanning the CAG repeats, shown in figure 17a. The sequence was mapped between markers D18S968 and D18S875 by STS content mapping on the YAC contig map.

A YAC 907-e-1 clone was transformed with the (CAG)₇ or (CTG)₁₀ fragmentation vector. The (CAG)₇ vector did not reveal the presence of any CAG repeat. Analysis of twenty-six (CTG)₁₀ fragmented YACs revealed that twenty-one of them had the same size of approximately 900kb. End Rescue was performed with KpnI on three fragmented YACS of this size. Sequencing revealed the nucleotide sequence shown in italics in Figure 18a. A Blast Search indicated the presence of an homology of this sequence with the GCT3G0I marker (GenBank accession number: G09484). The sequence spanning the CTG repeat was obtained from the GenBank Database. The sequence was mapped between markers 10R and WI-528.


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